

**Assessing treatment strategies to disrupt the vicious cycle of infection and  
inflammation in bronchiectasis**

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**This thesis is dedicated to my husband Ben- for his extreme patience, unfailing love and support- and to my parents, Stephen and Brenda, who are always there for me and for all their love and faith in me.**



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## **DECLARATION**

This thesis describes work undertaken in the University of Edinburgh's School of Medicine and Veterinary Medicine, Centre for Infectious Diseases and Centre for Inflammation Research, and the Department of Respiratory Medicine, Royal Infirmary of Edinburgh during my time as a Clinical Research Fellow from 2007 to 2009. The work described in this thesis has been my own and the writing of this thesis has been entirely my own undertaking. The majority of the work presented in this thesis has been published in peer reviewed academic journals and a list of both these and other relevant publications arising from this work constitute the Appendix. This work has not previously been submitted for a higher degree or other professional qualification.

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## **ABSTRACT**

**Background:** Non-cystic fibrosis bronchiectasis is a chronic debilitating disease. A perpetual cycle of excessive neutrophilic inflammation and chronic bacterial infection is thought to occur within the permanently damaged and dilated airways.

**Aim:** The aims of this thesis were to explore whether the vicious cycle of infection and inflammation in bronchiectasis can be disrupted, improving the clinical features of the disease and to identify potentially useful clinical markers for monitoring treatment response.

**Methods:** Patients with a radiological diagnosis of bronchiectasis and a clinical history of a daily productive cough, recurrent infective exacerbations and evidence of chronically infected sputum were recruited. The first study was a randomised crossover trial of regular chest physiotherapy in 20 patients assessing whether augmentation of the impaired mucociliary system can improve health related quality of life (HRQL) and the clinical features of bronchiectasis. The second study was a prospective cohort study of 32 infective exacerbations of bronchiectasis managed with two weeks' intravenous antibiotic therapy and assessed the impact of reducing bacterial infection in the airways during acute exacerbations on HRQL, sputum bacteriology, purulence and volume as well as lung function and systemic markers of inflammation. The third study was a randomised crossover trial of regular, long term nebulised gentamicin over 12 months in 57 patients exploring the impact on airways infection and inflammation, lung function, exercise capacity, HRQL and exacerbation frequency. The fourth study sought to identify potentially useful markers of response to chronic treatment strategies for stable disease by analysing markers common to the preceding two interventional studies to

assess their robustness and relevance, including sputum bacteriology and purulence, lung function, exercise capacity, systemic inflammatory markers and exacerbation frequency.

**Results:** Augmenting the impaired mucociliary system with regular chest physiotherapy improved HRQL and exercise capacity as well as increasing 24 hour sputum volume. Treatment of acute infective exacerbations improved patients' HRQL and exercise capacity as well as reducing 24 hour sputum volume, improving sputum purulence and decreasing systemic inflammation. Long term regular nebulised gentamicin significantly reduced sputum bacterial density and airways inflammation. Exercise capacity and HRQL improved with treatment and patients suffered fewer exacerbations. Parameters that may be useful in predicting a successful response to long term treatment strategies for clinically stable disease include an improvement of  $\geq 50$ m in the incremental shuttle walk test and eradication of pathogens from the sputum.

**Conclusion:** Augmentation of the impaired mucociliary system, effective treatment of acute infective insults and reducing chronic bacterial infection improves the clinical features of bronchiectasis and suppresses the vicious cycle of infection and inflammation. Potentially useful markers of a successful treatment response in the management of stable disease are exercise capacity and sputum bacterial clearance.

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## **CHAPTER ONE:**

### **INTRODUCTION**

## **1.1    Bronchiectasis**

Bronchiectasis (*Greek: bronkhia- “windpipe” and ektasis- “a stretching out”*): a chronic respiratory condition characterised by abnormal, permanently damaged and dilated bronchi with clinical manifestations of persistent or recurrent bronchial sepsis.

Rene Laennec first described bronchiectasis in a brief chapter entitled “On dilatation of the bronchi” in his treatise *Auscultation Mediate* in 1819 (Laennec, 1834). He commented on the clinical history and physical signs of four fatal cases and reported the morbid anatomy of the unifying condition: abnormal, permanent dilatation of the bronchi. Clark, Hadley and Chaplin in 1894 published the first living case series of 45 patients with the condition (Clark, 1894). They described symptoms of cough and sputum in their cases, briefly terming the chronic condition “fibroid disease of the lung”. However, it was not until over a century after Laennec’s original description that Sicard and Forestier in 1922 introduced the technique of injecting lipiodol into the trachea with concurrent radiological imaging that the anatomy of bronchiectasis could be studied with precision in living individuals (Sicard, 1922). This technique led to descriptions of different degrees of bronchial dilatation. In 1932 Moll described the varying appearances of the dilated bronchi as “(1) uniform, tubular or cylindrical; (2) fusiform or glove-finger; (3) globular or sacculated; (4) moniliform or bead-like.” (Moll, 1932). The clinical relevance of describing the appearance of the dilated airways was made apparent by Perry and King in their case series published in 1940, which evaluated the prognosis of the condition observing that patients diagnosed with “saccular or cystic” dilatation as opposed to “cylindrical” dilatation had a poorer prognosis in terms of both morbidity and mortality (Perry K.M.A, 1940). The

description and classification of the different degrees of bronchial dilatation is observer dependent and various interchangeable terms have been used over the years. However, in 1950 Reid reported on the findings of pathological and radiographic examination of 45 cases of bronchiectasis. Cylindrical (a uniform, minimal dilatation), varicose (irregular and greater dilatation) and cystic or saccular (progressive dilatation) types of bronchiectasis were described from the appearance of the bronchograms (Reid, 1950). These latter descriptions remain in use today for reporting the appearance of the bronchi on high resolution computed tomography of the chest, the now gold standard technique for diagnosing the condition.

## **1.2 Prevalence**

Population studies in bronchiectasis are limited perhaps given the diagnostic methodology required for an established diagnosis: morbid anatomy in the early 19<sup>th</sup> century, invasive bronchograms and chest radiographs in the first half of the 20<sup>th</sup> century to the current gold standard of computed tomography (CT) chest scans necessitating high dose radiation exposure. Some of the earliest UK prevalence reports were in the 1950s, with between 1.3 to 1.5 cases per 1000 reported in England and Wales in 1953 and 1956 from follow-up of abnormal chest radiographs during the tuberculosis eradication campaign (Wynn-Williams, 1953, Ministry of Health, 1947 & 1957). Throughout the following decades, indices of incidence and prevalence of bronchiectasis have used studies reporting hospital admission rates or thoracic surgical resection rates for bronchiectasis.

It is widely believed that there has been an overall decline in incidence of bronchiectasis with more effective treatment of childhood respiratory conditions, successful

vaccination programmes (particularly for measles and whooping cough) and effective treatment strategies for control and management of pulmonary tuberculosis. However, there is still little data available regarding the actual prevalence of bronchiectasis in the 21st century, again with no formal population studies to date. In 2005 a retrospective cohort study of US health claims by Weycker et al suggested a prevalence of bronchiectasis affecting 4.2 per 100 000 population aged 18-34 years and 271.8 per 100,000 population aged over 75 years of age (Weycker D, 2005). Twiss et al in New Zealand found an incidence of 3.7/100 000 in the paediatric population (Twiss et al., 2005b). More recently, 1,409 participants aged between 23 to 86 years old in a Korean health screening programme underwent a CT chest scan with 9.1% demonstrating evidence of bronchiectasis (Kwak et al., 2010). In primary care in the UK, a survey of respiratory disorders in a middle class suburban general practice serving 5840 patients identified 28 patients with bronchiectasis (0.47% of the practice's population) equating to approximately 12 cases per an average General Practitioner list of 2500 patients (Masters, 2010).

Bronchiectasis is recognised in the literature as an important problem in developing countries with Marostica emphasising the increased frequency of particular causative factors such as pneumonias, tuberculosis, postviral obliterative bronchiolitis in Latin America as factors that could be responsible for an increased frequency of bronchiectasis (Marostica and Fischer, 2006). Babayigit et al in their review of characteristics and aetiologic factors of bronchiectasis in a paediatric population in Turkey suggested that prevalence may reflect the socioeconomic conditions of the



particular population- where access to vaccinations is difficult and pulmonary infections are more frequent and severe (Babayigit et al., 2009).

Although it has not been a primary consideration, clinical studies of bronchiectasis to date have demonstrated an overall increased prevalence amongst females: 62.7% in Pasteur's study of causative factors (Pasteur et al., 2000); 69% in Nicotra's study of the characterisation of bronchiectasis in an ageing cohort (Nicotra et al., 1995); 53% in Ellis's study of prognosis and 55.8% in Keistinen's work (Ellis et al., 1981, Keistinen et al., 1997). Weycker and Shoemark found an increased prevalence amongst females of 63-68% (Weycker D, 2005, Shoemark et al., 2007). This observed difference in gender distribution was explored by Morrissey and Harper in 2004 (Morrissey and Harper, 2004) who proposed that both sociological and biological factors may be responsible. Sociologically, females may be more likely to seek medical attention for their symptoms and traditional societal roles may expose females to more aetiological risk factors, for example inhalation of smoke from open fire cooking in poorly ventilated areas or exposure to repeated viral infections while caring for young children. Such links are currently tenuous and would perhaps apply only to select geographic areas of the world. Biological factors may include the influence of the luteal phase of the menstrual cycle increasing airways reactivity which has been investigated to a limited extent in asthma as well as the different pulmonary anatomy in women compared with men: women have smaller lungs and with less muscle mass and strength, there is less pronounced expansion (and collapse) of the thorax than in men. Diseases that are frequently associated with or complicated by bronchiectasis may primarily be more

common in women, such as rheumatoid arthritis and Sjogren's syndrome (Symmons, 2002, Mavragani and Moutsopoulos, 2010).

### **1.3 Burden**

Morbidity in bronchiectasis can be measured in several ways, but perhaps of greatest socioeconomic importance is the morbidity associated with recurrent chest infections, necessitating days off work and utilisation of health care resources including hospital admissions. A retrospective study of 410 patients with bronchiectasis attending a respiratory outpatient clinic in Northern Ireland in 2003 randomly selected 100 patients and found that they had had a total of 321 clinic attendances over a 6 month period, with 39 patients requiring four or more reviews during this period. 48 of these patients had received four or more courses of antibiotics in the preceding 12 months and hospital admission for bronchiectasis had been necessary in 30 in the past year (Kelly et al., 2003). UK hospital admission data for 2007-8 found bronchiectasis to be the primary diagnosis in 1 in 1800 hospitalisations. A New Zealand study of factors predicting readmission and mortality in acute exacerbations of bronchiectasis found that the 152 patients included in their analysis had required a total of 307 hospital admissions over a 12 month period (Roberts et al., 2011). In a separate New Zealand study the Maori population were noted to have a high rate of hospitalisations for bronchiectasis of 30-50/100 000 (O'Neill M, 1995). US data for 1993-2006 found the average annual prevalence of hospitalisations due to bronchiectasis to be 16.5 per 100 000 population (Seitz et al., 2010). The latter study found an annual percentage increase in hospitalisations between 1993-2006 of 2.4% for men and 3.0% for women. The median

cost for inpatient care was US\$7,827(13-543,914). To date, no studies have explored the rate of primary care consultations for bronchiectasis patients.

More recently, studies have explored trends in mortality in bronchiectasis, although data are still limited. Bronchiectasis related deaths in England and Wales have shown a rise in absolute numbers of deaths per year from 797 in 2001 to 908 in 2007, a statistical increase in mortality of 3% per annum (Roberts and Hubbard, 2010). An analysis of factors related to mortality in bronchiectasis in 98 outpatients followed over a 4 year period found cumulative survival rates of 97% at one year, 89% at two years, 76% at three years and 58% at four years (Onen et al., 2007).

#### **1.4 Clinical features**

*“copious foetid sputum...marked constitutional symptoms...a social outcast...” Warner 1932 (Warner, 1932).*

As a clinical entity, the presentation of bronchiectasis is well described. Respiratory symptoms predominate with the defining feature of a persistent, productive cough present in 90.2-96% of patients (Nicotra et al., 1995, King et al., 2006a). Daily sputum is reported in approximately 75% of patients with mean±standard deviation daily volumes of 38±34mls of sputum found in a study of 103 patients with newly diagnosed bronchiectasis (King et al., 2006a). In clinical practice, sputum volume is variable depending on the extent of bronchiectasis and clinical stability of the patient. Sputum is often discoloured with a yellow or green purulence reflecting airways inflammation. An early study that included 14 patients with bronchiectasis and 7 patients with chronic bronchitis found that purulent sputum correlated strongly with increased concentrations

of neutrophil elastase ( $r=0.871$ ) and myeloperoxidase ( $r=0.882$ ) (Stockley et al., 2001). Haemoptysis is a variably reported feature with studies reporting frequencies of 26%-51.2% at diagnosis. Dyspnoea is also predominant with 60% of 103 newly diagnosed patients having a mean $\pm$ standard deviation MRC dyspnoea score of  $2.1\pm1.2$  units. Non-ischaemic chest pain, typically described as pleuritic or musculoskeletal may be described, with reported rates of between 19% and 46.3% (Nicotra et al., 1995, King et al., 2006a). One study found that 94.4% of chest pains reported in bronchiectasis were in an area associated with a bronchiectatic lobe (Munro et al., 1989). A major cause of morbidity is recurrent chest infections, with reports of  $2.4\pm1.6$  exacerbations per year in King's study of 103 patients with bronchiectasis (King et al., 2006a). Patients frequently suffer systemically with fatigue which, together with the chronic respiratory symptoms, adversely affects patients' health related quality of life (Wilson et al., 1997a). An earlier study also explored the impact of symptoms on patients' spouses and reported that 46% found their partner's cough to be distasteful and 29% reported that the symptoms interfered with their sexual life (Ellis et al., 1981).

Clinical signs in bronchiectasis may typically include crackles (69.9%-73%) and wheeze (21-34%). Clubbing is a recognised but infrequent clinical finding reported in 2-3% (King et al., 2006a). Frequently, physical examination may be entirely normal.

## **1.5    Diagnosis**

For almost a century after bronchiectasis was first described, the diagnosis of the disease was predominantly made on a clinical basis. Chest radiographs were insensitive and had poor specificity with potential diagnostic features including the presence of linear markings, bronchial wall thickening, patchy or confluent pulmonary shadowing, pleural thickening, lung collapse or volume loss or circular markings (Gudbjerg, 1955). The introduction of lipiodol (an iodine containing vegetable oil) into radiological practise by Sicard in the early 1920s and its later establishment in bronchopulmonary radiology by Sergent and Cottentot offered a more objective and reliable means of diagnosis with the injection of contrast into the bronchial tree (initially via intratracheal injection and later via bronchoscopy to produce a bronchogram) immediately prior to x-ray imaging of the chest (Sicard, 1922, Sergent E, 1923). The earliest signs of bronchial dilatation could be detected by bronchogram and were described in the literature as “small irregular fusiform shadows in connexion with medium sized bronchi” (Armand-Delille and Moncrieff, 1924). By 1935, it was recommended that

*“in every case of suspected bronchiectasis, radiology of the chest after lipiodol injection into the bronchial tree should form part of the routine investigation...” (Lloyd, 1935).*

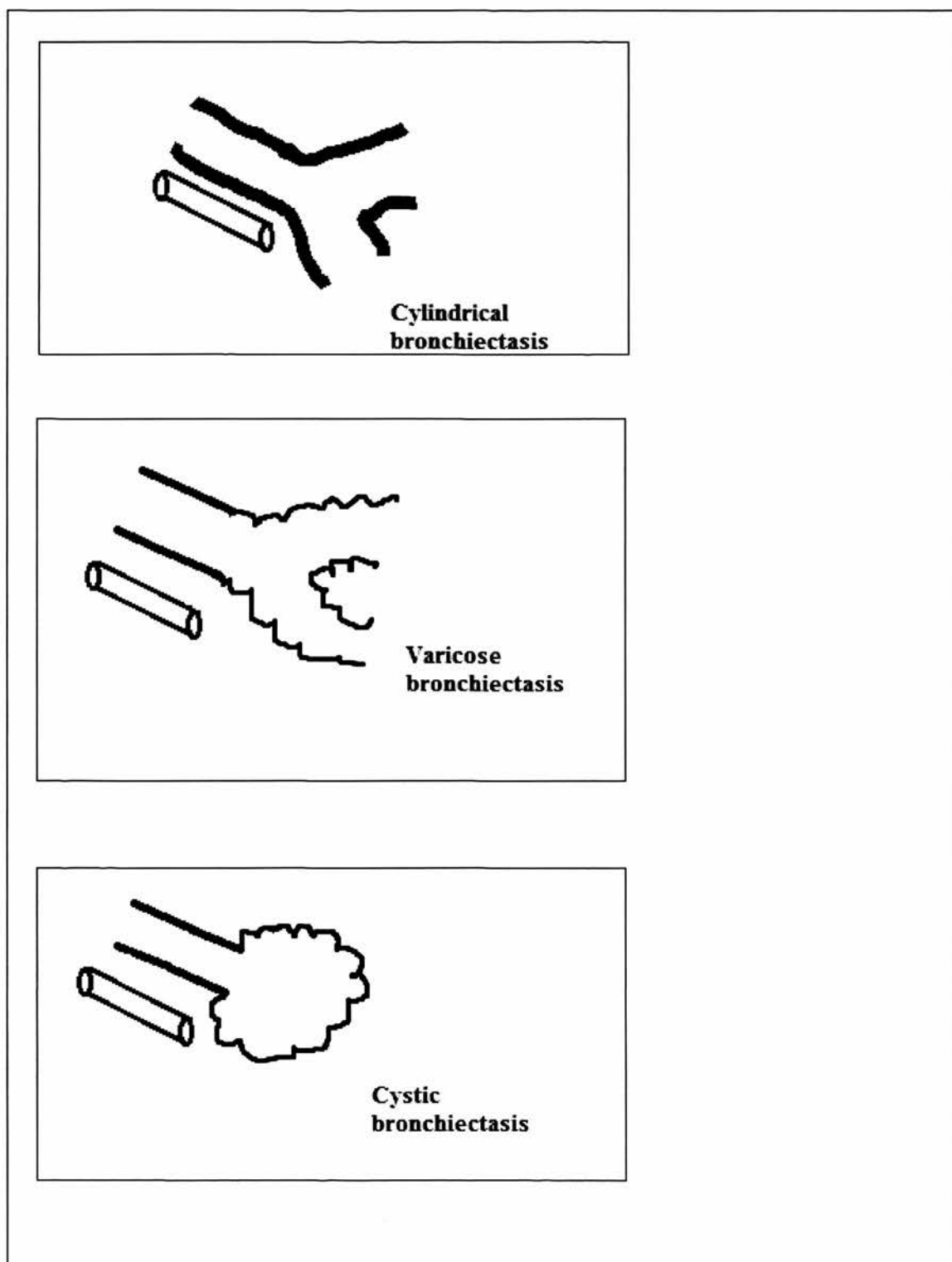
Later studies confirmed that plain chest radiographs were insensitive for diagnosing bronchiectasis in comparison with bronchograms (Currie et al., 1987a). The procedure was not without morbidity however, with potential risks including allergic reactions to the contrast or anaesthetic and temporary impairment of ventilation and diffusion. With the advent of more advanced radiological imaging techniques, the role of CT in the

diagnosis of bronchiectasis was investigated and later formally established such that it remains the 'gold standard' today to confirm clinically suspected bronchiectasis.

The role of CT in establishing the presence of bronchiectasis was initially proposed by in 1982 when 6 patients already diagnosed with bronchiectasis on the basis of chest radiograph (2 patients) and bronchogram (4 patients) underwent a CT scan confirming the chest radiograph and bronchogram findings (Naidich et al., 1982). CT signs of bronchiectasis were reported to include air-fluid levels, distended bronchi, linear arrays or clusters of cysts, dilated bronchi in the periphery of the lung and bronchial wall thickening (Naidich et al., 1982). The accuracy and reliability of CT in bronchiectasis was explored over the following decade, with the early studies comparing the accuracy of CT with the traditional bronchogram. An initial study found CT to be useful for cystic bronchiectasis but in cylindrical and varicose bronchiectasis CT scan did not detect disease in 5 cases diagnosed on bronchogram (Muller et al., 1984). A later study of cylindrical and mild varicose bronchiectasis diagnosed by bronchogram in 15 patients found that CT scans in these patients had a sensitivity of 79% and a specificity of 99%, indicating that CT is reliable but too insensitive (Phillips et al., 1986). Reasons for poorer sensitivity were explored and issues such as motion artefacts simulating the appearance of bronchiectasis (transmitted cardiac pulsations could mimic cystic bronchiectasis) and pathologic variations of disease such as mucoid impaction altering classical appearances of dilated bronchi were thought to contribute (Tarver et al., 1988). The development of thin section CT imaging markedly improved the sensitivity of the investigation with the rate improving to 84% in a study of 27 patients undergoing CT scans with 3mm high resolution images (Munro et al., 1990). A separate study of high-

resolution CT scans (HRCT) compared with bronchography found HRCT to be positive in 87 out of 89 segmental bronchi with bronchiectasis at bronchography and negative in 169 out of 170 segmental bronchi without bronchiectasis at bronchography giving a false negative rate of 2% and false positive rate of 1% (Young et al., 1991). HRCT assessment of the bronchi in bronchiectasis was also proven to have minimal inter- and intra-observer variation with good reproducibility of bronchial wall circumference (Desai et al., 1994). Such studies prompted the recommendation in 1993 that optimal evaluation necessitated selecting the thinnest possible slices of at least 1-2mm thickness for diagnosing bronchiectasis (McGuinness et al., 1993). HRCT imaging is now the gold standard investigation for diagnosing bronchiectasis with the defining feature being the presence of bronchial dilatation with the internal diameter of the bronchial lumen measuring greater than that of the adjacent artery, with the degree of dilatation typically categorised as tubular, varicose or cystic (Figure 1).

Figure 1. Categorisation of bronchiectasis.



Cylindrical, varicose and cystic bronchial dilatation in comparison with the adjacent bronchial artery.




Other radiological features that may be present in addition to those originally described by Naidich include emphysema, interlobular septal thickening (Sibtain et al., 2005) and a mosaic attenuation pattern due to small airways disease (Hansell et al., 1994). The role of HRCT scans beyond initial diagnosis in bronchiectasis has been explored in studies over the past few decades, including its discriminative value for aetiology and utility in assessing extent and progression of disease. Although there is no specific scoring system validated for use in bronchiectasis, modifications of a score for cystic fibrosis has been used in research studies. The Bhalla score includes the number of lobes affected and segments involved; degree of bronchial dilatation; severity of bronchial wall thickening; extent of mucus plugging; presence or absence of sacculations; presence and number of bullae; presence of emphysema and number of lobes affected; collapse or consolidation; mosaic perfusion; air trapping (Bhalla et al., 1991). Using a modification of the Bhalla score a study of 46 patients with bronchiectasis found that more severe disease on HRCT scan correlated with a poorer health related quality of life (Eshed et al., 2007). A separate study compared HRCT findings with pulmonary function tests in 94 patients (62 of whom had cystic disease and 32 cylindrical), finding that with cystic dilatation patients had a significantly lower FEV<sub>1</sub>, FVC, diffusion capacity of carbon monoxide and blood oxygenation than those with cylindrical dilatation (Alzeer, 2008). There have been few studies assessing whether serial CT imaging can be used to assess disease progression. The relationship between changes in serial CT scans and markers of pulmonary function in bronchiectasis in 48 patients with bronchiectasis over a median follow-up period of 28(6-74) months found that changes in the severity of bronchiectasis were seen more frequently on CT scan including areas of decreased attenuation, bronchial wall thickening and mucus plugging than in changes

in pulmonary function particularly the FEV<sub>1</sub> (Sheehan et al., 2002). Mucus plugging was the only radiological feature identified that correlated significantly with change in the FEV<sub>1</sub> (Sheehan et al., 2002). This study suggests that radiological disease progression may be evident within short time periods, but to date there are no long-term studies assessing regular radiological follow-up of disease.

The utility of CT scan in identifying the cause of bronchiectasis has been explored in a number of studies and at present it is thought to be of limited value particularly on an individual patient basis (Reiff et al., 1995) with poor inter-observer agreement on radiological findings in terms of likely cause (Lee et al., 1995, Cartier et al., 1999).

In addition to the radiological assessment of bronchiectasis, clinical and microbiological parameters are reviewed to help further define disease severity (Table 1).

Table 1. Assessment of disease severity.

Characteristic	Disease Severity		
	Mild	Moderate	Severe
			
<b>Sputum Colour</b>	Mucoid	Mucopurulent	Purulent
<b>24hr Sputum Volume</b>	<5mls		≥ 25mls
<b>Exacerbation Frequency</b>	< 3 per annum		≥ 3per annum
<b>Exacerbation Severity</b>	Oral antibiotics		IV antibiotics & adjunctive measures
<b>Sputum bacteriology</b>	Not chronically infected	Mixed flora or various pathogens (eg <i>H.influenzae</i> , <i>M.cattarhalis</i> , <i>S. pneumoniae</i> , <i>S.aureus</i> )	<i>Ps.aeruginosa</i> MRSA, enteric Gram negative organisms
<b>Affected lobes on CT scanning</b>	< 3 lobes		≥ 3 lobes
<b>Degree of bronchial dilatation</b>	Tubular dilatation	Varicose dilatation	Cystic dilatation

Clinical, microbiological and radiological indices used in disease severity assessment.

## **1.6 Aetiology**

Bronchiectasis is a pathological description of irreversibly damaged and dilated airways. It may be present as an independent entity or it may be an association of another disease process. Identifying the underlying aetiology of the bronchiectasis is important for formulating an appropriate management strategy.

In a study of 165 adult patients with a clinical and radiological diagnosis of bronchiectasis, an identifiable cause was found in 122 affecting management in 61 cases whose underlying aetiologies required specific therapeutic strategies. The underlying causes identified which affected management included primary ciliary dyskinesia, allergic bronchopulmonary aspergillosis, immune deficiency such as common variable immunodeficiency (CVID), ulcerative colitis, panbronchiolitis, mycobacterium infection (tuberculous or non-tuberculous infection), rheumatoid arthritis, aspiration and cystic fibrosis (Shoemark et al., 2007). An earlier study of 150 adult patients identified a causative factor for bronchiectasis in 47% of cases which had prognostic and therapeutic implications in 15% (Pasteur et al., 2000). Li et al examined a paediatric population group and found that identifying a cause for bronchiectasis led to a specific change in management in 56% (Li et al., 2005).

The presence of bronchiectasis as an association of another disease may have implications for the prognosis of the underlying disease. In rheumatoid arthritis, bronchiectasis is a well recognised feature that may be diagnosed either before or after the development of joint symptoms (Allain et al., 1997). In a study of 23 patients with rheumatoid arthritis and bronchiectasis, 18 were diagnosed with bronchiectasis at a mean of 24.7 years after onset of arthritic symptoms (Shadick et al., 1994). These

patients all had seropositive, severe nodular rheumatoid disease in comparison with patients who had bronchiectasis prior to the onset of arthritic disease which was typically seronegative, non-severe rheumatoid disease (Shadick et al., 1994). In a separate study of 32 patients over a 5.5 year follow-up period, the presence of both rheumatoid arthritis and bronchiectasis meant patients were five times more likely to die than matched controls with rheumatoid disease alone (Swinson et al., 1997). The presence of bronchiectasis in chronic obstructive pulmonary disease (COPD) is known to adversely influence clinical course of the disease. A study of 54 patients with COPD who underwent HRCT chest imaging found bronchiectasis to be present in 50%, with lower lobe bronchiectasis in 33.3% (Patel et al., 2004). COPD patients with bronchiectasis had greater levels of airways inflammation as measured by interleukin-8 and interleukin-6 in their sputum than those without bronchiectasis [for interleukin-8 (pg/ml) median(interquartile range) 3,939(3,173-5,528) versus 3,897(1,772-4,733),  $P=0.001$  respectively and for interleukin-6 (pg/ml) median(interquartile range) 113.2(20.1-218.9) versus 50.2(13.6-213),  $P=0.01$  respectively). The presence of lower lobe bronchiectasis was associated with a longer time to symptom recovery after an acute exacerbation (median of 12 days versus 10 days) (Patel et al., 2004). In interstitial lung disease, the presence of traction bronchiectasis on HRCT in patients with usual interstitial pneumonia adversely influenced prognosis, with increased mortality rates (hazard ratio 1.3, confidence interval 1.18-14.2) (Sumikawa et al., 2008).

A systematic investigation for causative factors or associated diseases is therefore important. Studies to date have published variable rates of identifiable aetiologies, with no cause found in up to 50% of patients (Kelly et al., 2003, Shoemark et al., 2007,

Pasteur et al., 2000). The rate of aetiological diagnosis varies perhaps due to differences in criteria for investigations as well as the population being studied- a higher proportion of post infective bronchiectasis as an identifiable cause may be seen in lower socioeconomic countries where undertreated infection or rate of tuberculous infection for example is greater. However, there are clearly defined causes or risk factors for bronchiectasis either as a single disease entity or as part of a syndrome that require exploration.

Aetiologies for bronchiectasis include: post-infective; aspiration/inhalation injuries to the airways; immunological- including both impaired immune defences and exaggerated immune responses; congenital syndromes; genetic diseases; systemic diseases and pulmonary diseases.

Table 2 gives the typical incidence of these causes and associations of bronchiectasis with their supporting diagnostic features.

Table 2. Aetiologies and incidences of bronchiectasis.

Aetiology	Incidence	Supporting diagnostic features
<p><b>Post infectious</b> (e.g. pneumonia, <i>Bordetella pertussis</i>, <i>Mycobacterium tuberculosis</i>, <i>Non-tuberculous mycobacteria</i>)</p>	29-42%	<ul style="list-style-type: none"> <li>History or radiological evidence of previous infection</li> </ul>
<p><b>Connective Tissue Disease</b> (commonly rheumatoid arthritis; also systemic sclerosis, systemic lupus erythematosus, relapsing polyarthritis, ankylosing spondylitis)</p>	3-6%	<ul style="list-style-type: none"> <li>History or clinical signs of connective tissue disease ± vasculitis</li> <li>Positive findings on autoimmune screen (e.g. rheumatoid factor, anti nuclear antibodies, antineutrophil cytoplasmic antibodies)</li> </ul>
<p><b>Allergic bronchopulmonary aspergillosis</b></p>	1-8%	<ul style="list-style-type: none"> <li>History of asthma</li> <li>Peripheral eosinophilia</li> <li>↑Total serum Immunoglobulin (Ig) E</li> <li>↑Specific IgE and IgG to <i>Aspergillus fumigatus</i></li> <li>+ve skin test reactivity to <i>Aspergillus fumigatus</i></li> <li>Fleeting infiltrates on chest radiograph or CT chest or proximal bronchiectasis on CT chest scan</li> </ul>

Aetiology	Incidence	Supporting diagnostic features
<b>Immunodeficiency</b> (for example Common Variable Immunodeficiency, X-linked agammaglobulinemia, IgA deficiency, IgG subclass deficiency)	1-8%	<ul style="list-style-type: none"> <li>• Variable deficiencies possible [IgG (including IgG subclasses), IgA, IgM]</li> <li>• In IgG subclass 2 deficiency, history of recurrent infections with Gram+ve encapsulated organisms</li> <li>• Low baseline specific antibody levels and poor antibody response against Tetanus toxoid and the polysaccharide capsules of <i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> type B</li> </ul>
<b>Cystic Fibrosis</b>	1-4%	<ul style="list-style-type: none"> <li>• Symptoms of malabsorption, male infertility</li> <li>• Upper lobe bronchiectasis on CT chest scan</li> <li>• Persistent culture of <i>Staphylococcus aureus</i> in sputum</li> <li>• Positive cytogenetics for cystic fibrosis transmembrane regulator receptor mutations</li> <li>• Positive sweat test</li> </ul>
<b>Ciliary Defect</b> (e.g. primary ciliary dyskinesia)	1-10%	<ul style="list-style-type: none"> <li>• History of chronic upper respiratory tract problems, otitis media, male infertility</li> <li>• Positive saccharin taste test (saccharin not tasted after 60 minutes)</li> <li>• Abnormal ciliary beat pattern ± frequency on nasal brushings</li> </ul>
<b>Inflammatory Bowel Disease</b> (Ulcerative colitis, Crohn's disease)	1-3%	<ul style="list-style-type: none"> <li>• History or clinical signs</li> <li>• Positive pathological features on colonoscopy</li> </ul>



Aetiology	Incidence	Supporting diagnostic features
<b>Aspiration/Inhalation of foreign body</b>	1-4%	<ul style="list-style-type: none"> <li>• History of aspiration</li> <li>• Localised (single lobe) bronchiectasis</li> <li>• Positive findings at bronchoscopy</li> </ul>
<b>Idiopathic</b>	26-53%	<ul style="list-style-type: none"> <li>• Above causes excluded</li> </ul>

### *Post-infectious*

An infective insult to the airways is thought to be sufficient to cause bronchiectasis. Exposure to infective pathogens causing airway wall inflammation and structural damage may follow bacterial pneumonia, infection with *Bordetella pertussis*, infection with Mycobacterial disease (both tuberculous and non-tuberculous) and viral infections (such as measles, adenovirus, rhinovirus and respiratory syncytial virus). Symptoms may occur soon after the original infection or there may be a significant delay between the original infective insult and presentation with chronic respiratory symptoms. The closer the occurrence between insult and presentation, the greater likelihood that the infection is responsible for the bronchiectasis. Post-tuberculous bronchiectasis is typically in a segmental or lobar distribution. Non-tuberculous mycobacterial infection may be implicated before or after the diagnosis of bronchiectasis, the most frequent isolate being *Mycobacterium avium* complex (Marras and Daley, 2002, Field and Cowie, 2006, Waller et al., 2006, Winthrop et al., 2010, Wickremasinghe et al., 2005). There is a greater frequency of non-tuberculous mycobacterial infection and bronchiectasis in females and a classic syndrome of isolated middle lobe or lingular bronchiectasis due to non-tuberculous mycobacterial infection in females often described “the Lady Windermere syndrome”. The term “Lady Windermere syndrome” was proposed by Reich and Johnson following their case reports of pulmonary *Mycobacterium avium* infection and isolated middle lobe or lingular bronchiectasis in females (Reich and Johnson, 1992). They proposed that cough suppression, likely to be more common in females for societal purposes, led to inadequate clearance of dependant lobes prolonging the exposure of the airways in such lobes to infection and

consequent development of bronchiectasis. Oscar Wilde's play "Lady Windermere's fan" described such a female character inhibited by society to cough effectively.

#### *Inhalational Injuries and aspiration*

Aspiration of a foreign body causes a mechanical obstruction in the affected airways with accumulation of distal secretions. The prolonged exposure of such secretions to the airways' walls leads to chronic inflammation, structural wall damage and bronchiectasis. Intrinsic airway tumours (both benign and malignant) may theoretically through the same mechanism lead to the development of bronchiectasis, although this is exceptionally rare in series studied to date.

Gastro-oesophageal reflux disease is acknowledged as a potential risk factor for bronchiectasis and the association of *Helicobacter pylori* (*H.pylori*) infection in disease pathogenesis has been explored. An early study of 100 patients with bronchiectasis found 76% to be seropositive for IgG to *H.pylori* compared with a 54.3% prevalence in 94 healthy controls (Tsang et al., 1998b). In the patients with bronchiectasis, *H.pylori* seroprevalence was significantly higher in those who expectorated more than 5mls of sputum per 24hour period. A smaller study of 46 patients found a seroprevalence of 46% but on immunohistochemical staining of bronchial mucosa there was no evidence of presence of *H.pylori* (Angrill et al., 2006). A later study of 26 patients found no evidence of *H.pylori* in bronchial washings using a DNA polymerase chain reaction technique and also found no significant difference in serum IgG levels for *H.pylori* compared with healthy controls (Gulhan et al., 2007). The role of *H.pylori* in pathogenesis of bronchiectasis remains unclear but the earlier studies suggest that there may be an association. The prevalence of gastro-oesophageal reflux disease in patients

with bronchiectasis is unknown although there has been one study of patients with non-tuberculous mycobacterial infection that observed a 26% prevalence in patients with nodular bronchiectasis and non-tuberculous mycobacterial infection (Koh et al., 2007).

### *Immunological*

Impairment of the host defences may lead to recurrent bacterial infections and airway damage. Host defences may be impaired directly in the airways as with ciliary defects that inhibit the normal function of the mucociliary clearance mechanism or the presence of systemic immune deficiency may predispose to recurrent infection and airway wall damage.

Primary ciliary dyskinesia is usually an autosomal recessive disorder causing ultrastructural defects in the cilia affecting normal motility and is characterised by upper and lower respiratory tract infections (Noone et al., 2004). Kartagener's Syndrome describes the classic triad of situs inversus, bronchiectasis and chronic sinusitis due to a congenital reduction or absence of ciliary function (Kartagener, 1933).

Primary antibody deficiency syndromes have been associated with bronchiectasis for over half a century (Busse et al., 2007). Common variable immune deficiency (CVID) is associated with bronchiectasis in 16% - 68% of cases and 7% - 20% of X-linked agammaglobulinaemia (XLA) cases are associated with bronchiectasis (Thickett et al., 2002, Martinez Garcia et al., 2001, Aghamohammadi et al., 2010). Less commonly, bronchiectasis may complicate patients with immunoglobulin A (IgA) deficiency, although this tends to happen when other associated autoimmune defects occur (Aghamohammadi et al., 2009). Studies have also explored the role of immunoglobulin

G (IgG) and IgG subclass deficiencies in bronchiectasis. An early study of 65 patients aged 10-74 years with bronchiectasis of unknown aetiology found that 48% had IgG deficiency (61.3% were deficient in IgG subclass 2) all of whom had an impaired antibody response to *Haemophilus influenzae* Type B (De Gracia et al., 1996). A separate study also observed an impaired antibody response to *Haemophilus influenzae* Type B and *Streptococcus pneumoniae* in patients with IgG subclass 2 deficiency (Vendrell et al., 2005). Other studies however, have suggested that such isolated antibody subclass deficiency is an uncommon cause or association in adults aged over 40 years presenting with bronchiectasis with incidences ranging from 6.6% - 23.2% (Hill et al., 1998, Stead et al., 2002, King et al., 2006b). Considering an underlying immunodeficiency disorder is essential, particularly in those with opportunistic infections or indeed persistent severe infections as disease progression may be prevented with effective immunoglobulin replacement. The current UK national guidelines consensus expert opinion advocates functional testing to peptide and polysaccharide antigens as well as measurement of serum IgG, IgA and IgM in routine practice (Pasteur et al., 2010).

Secondary immune deficiency syndromes have recently become increasingly recognised as risk factors for bronchiectasis. Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Disease (AIDS) are frequently associated with both recurrent lower respiratory tract infections and with more severe infection with opportunistic organisms and viruses such as non-tuberculous mycobacteria and *Pneumocystis jiroveci* pneumonitis which in a study of paediatric HIV patients have been reported as significant risk factors for the development of bronchiectasis (Berman et al., 2007).

Exaggerated immune responses exposing the airways to excessive inflammation and damage can also lead to bronchiectasis. Allergic bronchopulmonary aspergillosis (ABPA) is a complex clinical condition that arises from an allergic immune response to *Aspergillus fumigatus*. It is defined by clinical, laboratory and radiologic features including active asthma, elevated levels of total serum IgE, serum eosinophilia and fleeting pulmonary parenchymal opacities on the chest radiograph (Patterson and Strek, 2010). More advanced disease is complicated by bronchiectasis which is classically central with peripheral sparing on HRCT scan (McGuinness and Naidich, 2002). Institution of glucocorticoid therapy may help to control the immunologic response and may limit the progressive damage (Patterson and Strek, 2010).

### *Congenital Syndromes*

Congenital abnormalities causing bronchiectasis are rare but important to diagnose given that most are associated with other complications. Specific congenital causes include Mounier-Kuhn syndrome and the Williams-Campbell syndrome.

Mounier-Kuhn syndrome (tracheobronchomegaly) was first described in 1932 and is a rare congenital abnormality characterised by marked thinning of the smooth muscle layer in the trachea and main bronchi as well as atrophy or complete absence of the elastic fibres (Mounier-Kuhn, 1932). The airways are therefore flaccid and markedly dilated on inspiration with complete collapse on expiration, leading to increased dead space and diminished clearance of secretions.

Williams-Campbell syndrome (bronchomalacia) was described in 1960 and refers to the complete absence of bronchial wall cartilage (Williams and Campbell, 1960). This

produces a mechanical abnormality with marked dilatation of the proximal airways on inspiration and complete collapse with expiration again leading to ineffective clearance of secretions and the development of bronchiectasis.

Pulmonary sequestration is a congenital abnormality that may lead to the development of bronchiectasis (Savic et al., 1979). There are two forms: extralobar (where the primitive tissue is enclosed within its own pleural sac and venous drainage is via the systemic venous system) and intralobar (where the primitive tissue is invested by the normal visceral lung pleura and venous drainage is via the pulmonary veins). In both forms there is no communication with the normal tracheobronchial tree and are instead aerated via the pores of Kohn. These patients are vulnerable to multiple respiratory infections and are therefore exposed to the risk of developing bronchiectasis.

### *Genetic Diseases*

Perhaps the most well defined, researched and specifically managed cause of bronchiectasis is cystic fibrosis (Fanconi G, 1936, Anderson, 1938). It is an autosomal recessive condition with a genetic mutation (the most common genotype being homozygous deltaF508) causing a reduction in the activity of the chloride channel the cystic fibrosis transmembrane conductor receptor (CFTR) (Riordan et al., 1989). The defect impairs electrolyte transport, causing viscous secretions to accumulate in the bronchial lumen, leading to disruption of the normal mucociliary clearance system. Recurrent respiratory tract infections occur, cystic bronchiectasis develops and progressive respiratory failure ensues. Extrapulmonary manifestations of the disease may include pancreatic insufficiency or failure, gastrointestinal disease, male infertility and raised sweat chloride levels. The condition is now screened for in all newborns in

the UK but may still present in later life and should be considered as a diagnosis. This form of bronchiectasis is studied and managed as an entirely distinct clinical entity.

More recently, Fajac et al proposed that a genetic defect causing dysfunction of the epithelial sodium channel protein ENaC could be responsible for development of non-cystic fibrosis bronchiectasis (Fajac et al., 2008). They analysed the genes ENaC $\beta$  and ENaC $\gamma$  in 55 patients with idiopathic bronchiectasis of whom 38 had functional evidence of a defective sodium channel and found eight patients had an ENaC mutation. Further studies are needed to explore this potential genetic cause of bronchiectasis.

### *Systemic Diseases*

Bronchiectasis is a recognised association of several systemic diseases, in particular connective tissue diseases and inflammatory bowel disease.

The association of rheumatoid arthritis and bronchiectasis was reported by Walker in 1967 who found the incidence of bronchiectasis to be 3.1% (Walker, 1967). In a later study of 68 patients with rheumatoid arthritis who underwent HRCT chest scans, the most frequent abnormality observed was bronchiectasis (30%) and 8% of these patients had no respiratory symptoms (Cortet et al., 1995). The association is well recognised however, the mechanism of development of bronchiectasis in patients with rheumatoid arthritis is unknown.

Primary Sjogren's syndrome is a chronic inflammatory autoimmune disorder characterised by a triad of keratoconjunctivitis sicca, xerostomia and in more than 50% a connective tissue disorder. A recent study of 507 patients with Primary Sjogren's syndrome who underwent HRCT chest scans found bronchiectasis present in 50



patients, 41 of whom had no other identifiable cause for the bronchiectasis (Soto-Cardenas et al., 2010). Although this study sought to further characterise the patients with bronchiectasis and immunologically found a lower frequency of anti-Ro/SSA antibodies and a higher frequency of anti-smooth muscle antibodies compared to those patients without bronchiectasis, the pathogenesis of bronchiectasis development in the syndrome is unknown.

Ankylosing spondylitis is a multisystem disorder with both articular and extra-articular features. Pulmonary complications include apical fibrobullous disease that is susceptible to supra-added infection. Infection in structurally abnormal lung can predispose to bronchiectasis. A study of 52 patients with ankylosing spondylitis and no respiratory symptoms who underwent HRCT scans found bronchiectasis present in 8% (Sampaio-Barros et al., 2007).

Associations between other connective tissue disorders such as systemic lupus erythematosus (SLE) and relapsing polychondritis with bronchiectasis are documented and should be considered as an aetiological risk factor or cause (Fenlon et al., 1996, Tillie-Leblond et al., 1998).

Inflammatory bowel diseases are associated with bronchiectasis. The association was first recognised by Kraft in 1976 and is more commonly reported with ulcerative colitis than Crohn's disease (Kraft et al., 1976). Although the immunopathological basis for the development of bronchiectasis is unknown, it is thought that the relationship may be due to the common origin of bowel and lung from the primitive gut (Higenbottam et al., 1980). Studies to date have shown that patients with inflammatory bowel disease and bronchiectasis have airways that are typically inflamed and patients expectorate

purulent sputum but bacterial colonisation is infrequent (Moles et al., 1988, Bonniere et al., 1986). Studies have suggested that inhaled or systemic corticosteroids may be beneficial in therapeutic management (Camus et al., 1993, Mahadeva et al., 2000).

Yellow nail syndrome was first described in 1964 and is characterised by the triad of dystrophic yellow coloured nails, lymphoedema and chronic respiratory manifestations (Samman and White, 1964). A study of 41 consecutive cases of patients with yellow nail syndrome found bronchiectasis in 44% (Maldonado et al., 2008). The pathogenesis for the development of bronchiectasis in yellow nail syndrome has not been established.

### *Pulmonary Diseases*

Bronchiectasis is frequently found in association with other pulmonary conditions, particularly COPD, ILD and possibly  $\alpha$ -1 antitrypsin deficiency. Bronchiectasis is typically recognised as a complication in COPD and ILD, however in alpha-one antitrypsin deficiency there is debate as to whether bronchiectasis is a complication of the condition or caused by the disease.  $\alpha$ -1 antitrypsin is an antiprotease responsible for control of protease activity from lysed phagocytes. The condition classically causes panacinar and basal emphysema and is also associated with cirrhosis of the liver. The association of the deficiency as a causative factor for bronchiectasis is contentious as two large case series found no radiological evidence of bronchiectasis on the chest radiographs of patients with  $\alpha$ -1 antitrypsin deficiency (Hutchison et al., 1983, Brantly et al., 1988). However, other radiological studies have contested this finding, observing bronchiectasis in 43% (King et al., 1996) and 95% (Parr et al., 2007) of  $\alpha$ -1 antitrypsin deficient patients and a post-mortem study found evidence of bronchiectasis in 15% of

42 cases examined (Tomashefski et al., 2004). It should be considered as a diagnosis when patients have basal emphysema.

Traction bronchiectasis is a radiological feature of idiopathic interstitial pneumonias, particularly usual interstitial pneumonia and non-specific interstitial pneumonia (Lynch et al., 2005). In a recent study of 146 patients with fibrotic interstitial idiopathic pneumonias, increasingly severe traction bronchiectasis was found to be a factor indicative of increased mortality irrespective of the high resolution CT chest radiological pattern and extent of disease (Edey et al., 2011).

Radiological studies of patients with COPD have found variable rates of bronchiectasis. Two studies of patients diagnosed with COPD in primary care found radiological evidence of bronchiectasis in 29% and 50% respectively although the studies did not explore whether these patients had any alternative cause for the bronchiectasis (O'Brien et al., 2000, Patel et al., 2004). The latter study found that in those patients with COPD and bronchiectasis there were higher sputum concentrations of interleukin-8 and time to recovery from an exacerbation of COPD was longer than in those without bronchiectasis (Patel et al., 2004). More recently, a study of 92 patients with COPD (51 with severe COPD and 41 with moderate COPD) found that 53 patients had bronchiectasis (Martinez-Garcia et al., 2011). The study identified the presence of severe airflow obstruction, isolation of a potentially pathogenic organism in the sputum and at least one hospitalisation with an exacerbation of COPD in the 12 months preceding entry to the study to be independent variables associated with the presence of bronchiectasis. The association of COPD and bronchiectasis requires further study.

### *Idiopathic*

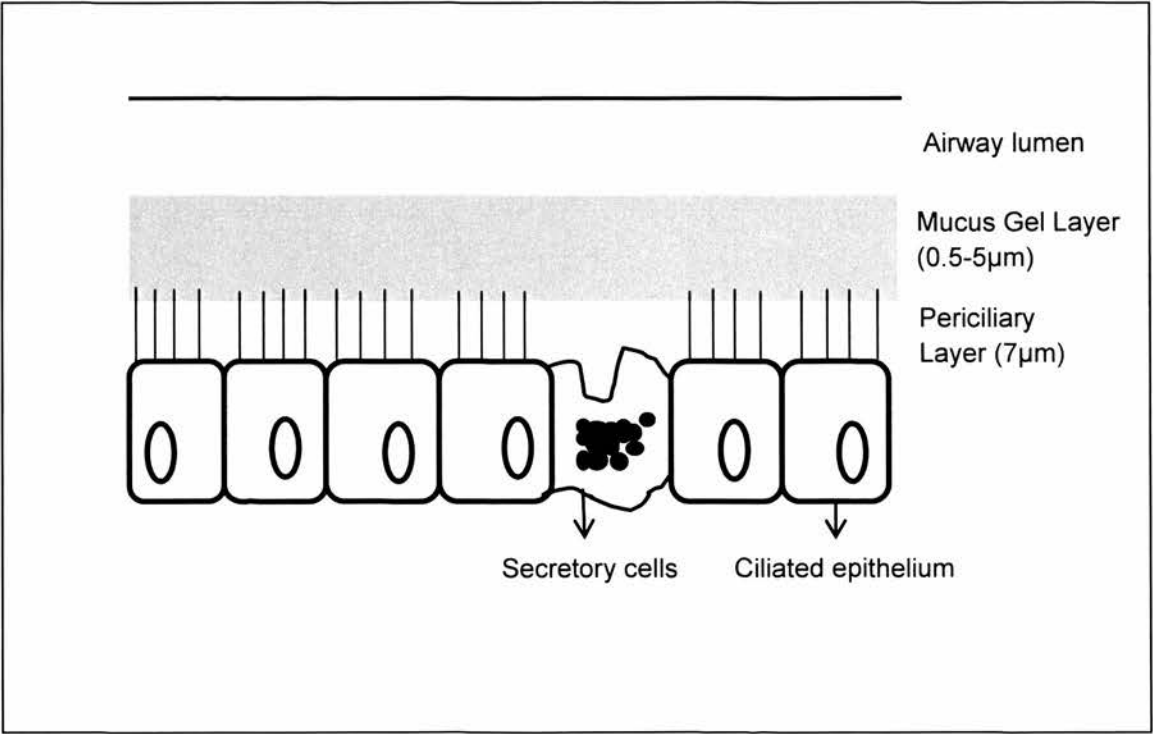
In up to 50% of patients no clear aetiology for the bronchiectasis is found. Such patients are described as having idiopathic bronchiectasis. It is however, a diagnosis only after major aetiologies and in particularly aetiologies that demand specific therapeutic strategies are investigated for and excluded.

Irrespective of the aetiology for bronchiectasis developing, the end result is irreversibly damaged and dilated airways. The usual pulmonary defence system is disrupted which has significant consequences for airway integrity with subsequent bacterial invasion and inflammation causing the perpetual symptoms and significant respiratory morbidity.

## **1.7**    **Airway host defence and damage**

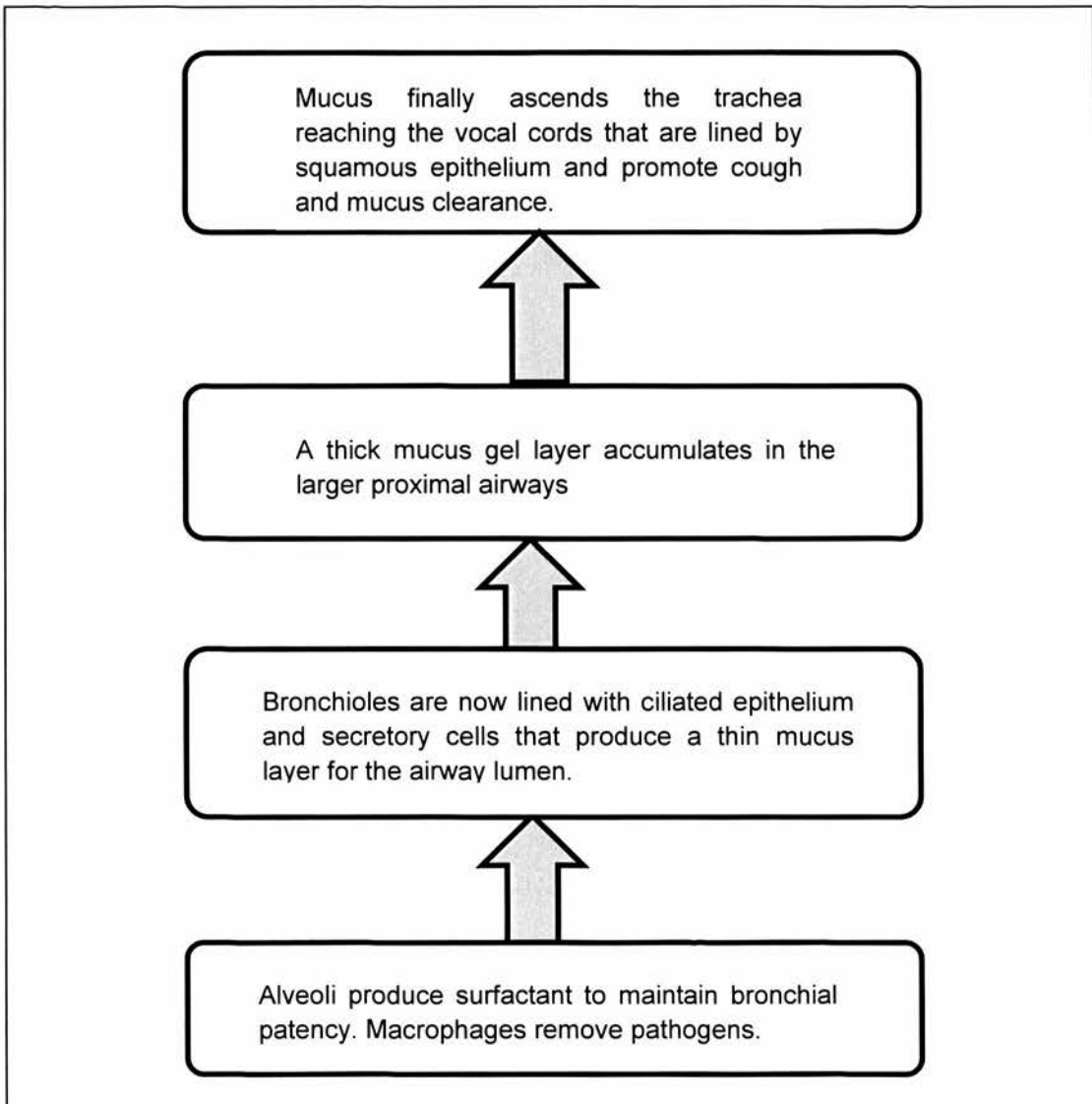
The airway mucociliary clearance system is a key component of primary host defence in the lungs, responsible for maintaining airway sterility and health. The system comprises of a mucus gel layer that lines the airway lumen trapping pathogens, inhaled toxins and cellular debris as it is swept by beating cilia from the distal airways to the proximal airways for expectoration and clearance (Figure 2).

Figure 2. The normal mucociliary clearance system.



In health, the respiratory tract is lined by equal numbers of ciliated columnar epithelial cells and secretory cells. The epithelial cells each have 200 cilia, approximately 7 $\mu$ m length that rest in a thin layer of periciliary fluid. The cilia beat in a 2 stage process, an effective beat where their tips engage with the airway mucus sweeping it through the airways and a recovery beat in the opposite direction where their tips disengage and recover (Purkinje, 1835, Lucas, 1934). The secretory cells release mucins, antimicrobial molecules (such as defensins and lysozymes), immunomodulatory molecules (cytokines) and protective molecules which become integrated within the extracellular mucus gel that lines the airway lumen. The airway mucus is composed predominantly of water (97%) and mucins (3%) and accumulates as it is transported through the larger airways, trachea and pharynx for expectoration (Figure 3).

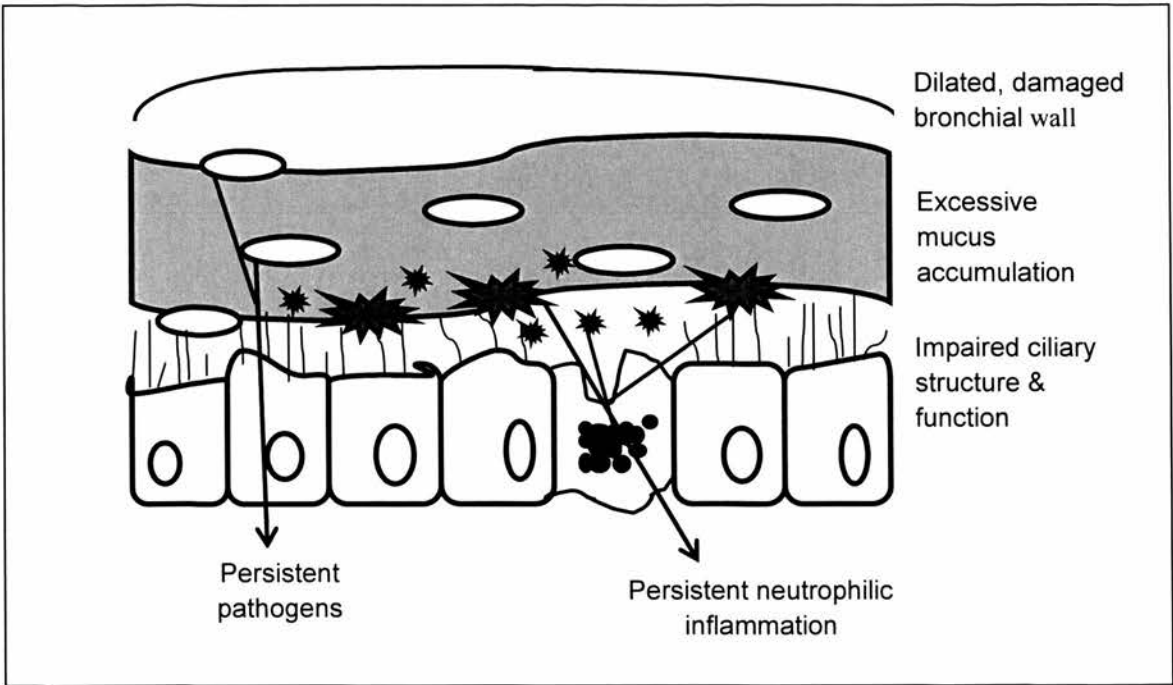
Figure 3. Mucus transport through the airways.





The integrity and efficiency of this system is crucial for airway sterility: a deficient mucus layer leaves the airways vulnerable to injury; disorders of ciliary structure or function impair clearance of the mucus and excessive mucus accumulation causes the airways to be persistently exposed to toxic insults and pathogens. In bronchiectasis, the airways are permanently damaged and the normal mucociliary mechanism is believed to be impaired (Figure 4).

Figure 4. The impaired mucociliary system in a bronchiectatic airway.



### *Impaired mucociliary clearance*

Tracheobronchial clearance studies provided the initial evidence for impaired mucociliary clearance in bronchiectasis. In 1972, Lourenco et al studied the distribution and clearance of inhaled aerosols in 14 patients with bronchiectasis (Lourenco et al., 1972). 8 patients were noted to have a central deposition of particles compared with the diffuse distribution of inhaled particles in healthy subjects. It was also observed that fewer particles were deposited in the saccular bronchiectatic areas than in the areas without bronchiectasis when reviewed with the patients' original diagnostic bronchography. These patients reported more severe symptoms and had more marked airways obstruction on lung function testing. All patients in the study were noted to have abnormalities in clearance of the inhaled aerosols, with a particularly slow rate immediately following inhalation. These findings were confirmed when similar observations were made in a study of 12 patients with bronchiectasis and 10 healthy controls (Currie et al., 1987b). In patients with bronchiectasis, the particles from an inhaled radio-aerosol were deposited in the more proximal airways which the authors postulated was due to deficiencies in airway patency. Tracheobronchial clearance of the particles in the 6 hours following inhalation was significantly slower in the bronchiectasis group despite a significantly greater cough frequency in these patients [36 coughs versus 0 coughs in the healthy subjects]. The relationship between impaired mucus clearance and clinical features was demonstrated in a study of 21 patients with bronchiectasis (Svartengren et al., 1986). At 2 hours following inhalation of labelled Teflon particles, patients with bronchiectasis retained  $65 \pm 27\%$  compared with  $36 \pm 22\%$  in healthy subjects and with more impaired clearance, there was found to be a greater

presence of generalised airway symptoms, more continuous symptoms and a longer history of the disease. A later study of mucociliary clearance in 20 patients with bronchiectasis demonstrated a diminished or uneven distribution of inhaled radiolabelled aerosol particles in bronchiectatic regions of the lung and in 19 (95%) of the patients transport of the aerosol from the bronchiectatic regions was abnormal (Isawa et al., 1990). The pattern of abnormal clearance from these areas of bronchiectasis included total stasis (12 patients), regurgitation backwards within the airway (14 patients), straying (9 patients) or a spiral movement (one patient). Abnormal clearance was further accentuated by cough, with increased regurgitation and straying of particles within the bronchiectatic regions observed.

#### *Abnormal ciliary structure*

Normal ciliary structure is integral to the function of the mucociliary clearance system. Each cilium is composed of an axoneme which consists of a circular formation of 9 pairs of microtubules surrounding 2 single, central microtubules. Each pair of microtubules is connected by nexin links and spokes radiate inwards from each pair to the central microtubules to maintain the integrity of the axoneme. Each pair of microtubules has small inner and outer dynein arms which contain adenine triphosphatase (ATP) which is hydrolysed to ultimately facilitate the movement of the microtubule pairs. Key features of normal ciliary structure are the “9+2” formation of the axoneme and the parallel orientation of each cilia’s 2 central microtubules which is essential for coordinated ciliary movement. Studies of ciliary abnormalities in bronchiectasis are limited, mainly involving patients with primary ciliary dyskinesia (proven genetic deficiencies causing recognised ultrastructural abnormalities of the

cilia) rather than those with idiopathic or other aetiologies for their bronchiectasis. Secondary ciliary dyskinesias include ciliary abnormalities responsible for dysfunctional movement that are present independent of any specific genetic ciliary defects. Ciliary anomalies that have been recognised in bronchiectasis include abnormalities of the orientation of the central microtubules, loss of the 9+2 formation, deficiencies or absence of the dynein arms and abnormalities of the nexin links in the pairs of microtubules (Sturgess et al., 1979, Cowan et al., 2001). An early study observed either partial or complete loss of the dynein arms within the ultrastructure of the cilia in 20 Polynesian patients with bronchiectasis (Waite et al., 1981). A later study including 15 patients with idiopathic bronchiectasis and proven normal ciliary ultrastructure and beat frequency observed that the beat direction was disorientated, correlating with impaired mucociliary clearance (Rayner et al., 1995). In a study of 133 patients with bronchiectasis, patients with more severe disease (defined as FEV<sub>1</sub> and FVC both <60% predicted and ≥4 lobes affected) had a significantly lower angle of orientation of the central microtubules than those with non-severe disease. The central orientation angle of the microtubules correlated with reduced ciliary beat frequency and the percentage of cilia with ultrastructural defects. However, the angle of the microtubules was not found to have any relationship in terms of aetiology of the bronchiectasis or colonisation with *Pseudomonas aeruginosa*, questioning the clinical relevance of this aspect of ciliary function (Tsang et al., 2005c). A prospective systematic evaluation of ciliary defects and clinical parameters in 152 patients with idiopathic bronchiectasis compared with 127 healthy controls found a greater percentage of central and peripheral microtubule defects [odds ratio 14.4(95% confidence interval 5.6-36.8), typically additional peripheral microtubules (disrupting

the 9+2 formation) in the bronchiectasis group. The percentage of cilia with central microtubular defects correlated with increased 24hour sputum volume and reduced FEV<sub>1</sub>, suggesting that structural abnormalities of the cilia may have an important clinical impact (Tsang et al., 2005b).

#### *Abnormal ciliary function*

In order to ensure effective mucus transport, the cilia must beat at a normal rate, in a consistent direction and in a coordinated fashion (Eliezer et al., 1970). Studies have used ciliated nasal epithelium as a non-invasive means of assessing ciliary function in the respiratory tract. Ciliary beat frequency is significantly slower in bronchiectasis with an early study of 10 patients with bronchiectasis observing the mean $\pm$ SD ciliary beat frequency to be 12.8 $\pm$ 1.3beats/second compared with 14.2 $\pm$ 0.7beats/second in healthy ciliated nasal epithelium ( $P<0.02$ ) (Rutland and Cole, 1981). This finding has been confirmed in a more recent study of 152 patients with idiopathic bronchiectasis which found ciliary beat frequency to be significantly slower than healthy controls (Tsang et al., 2005b). In vitro studies have sought to explore potential causes for reduced ciliary beat frequency and have observed that cilia from sites of purulent secretions beat more slowly (13.6Hz in bronchiectasis compared with 14.3Hz in cilia not exposed to purulent secretions) (Wilson et al., 1986). Potential reasons for a reduced ciliary beat frequency in areas of purulent secretions have been investigated and may be broadly classified as either host factors or pathogen factors. Host factors include exposure to excessive release of inflammatory mediators from persistent neutrophilic inflammation in the airways and pathogen factors include exposure to potentially toxic bacterial products from persistent colonisation within the damaged airway lumen. Early in vitro work

observed that the proteolytic enzyme elastase released by neutrophils is capable of inhibiting normal ciliary activity and damaging respiratory epithelium (Tegner et al., 1979). The purulent sputum in bronchiectasis contains excessive quantities of elastase due to the persistent neutrophilic airways inflammation. Such purulent sputum from patients with bronchiectasis has been shown to slow ciliary beat frequency on normal ciliated nasal epithelium in healthy controls from 13.4beats/second to 6.78beats/second over a 6 hour exposure period. The addition to the sputum of an elastase inhibitor ( $\alpha$ 1-antitrypsin) resulted in no significant change in ciliary beat frequency over the same time period, suggesting that elastase activity is an independent factor affecting ciliary function (Smallman et al., 1984). The effect of elastase on ciliary function and ciliated epithelium has been shown in vitro to increase with increasing concentrations of elastase in the sputum with progressive disruption of the epithelium and slowing of ciliary beat frequency at higher levels (Amitani et al., 1991). The effect of other inflammatory molecules on ciliary function in bronchiectasis is unknown and needs further study. The effect of bacterial products on ciliary function has been investigated in vitro. Exposure of ciliated nasal epithelium to the supernatants of *Pseudomonas aeruginosa* and *Haemophilus influenzae* over a 4 hour period was associated with significant slowing of ciliary beat frequency and a disorganised beating pattern. A dose related effect was also observed and it may be that the release of a factor or factors by these organisms complements the effect of human neutrophil elastase on ciliary inhibition (Wilson et al., 1985). The hypothesis that both host factors and bacterial factors may together affect ciliary function was further supported in a study of 20 patients with bronchiectasis, of whom 13 had a slower ciliary beat frequency. In these 13 patients, two distinct patterns of epithelial damage and ciliary function were

observed: the first pattern involved a gradual disruption of the epithelium and was inhibited by the addition of the elastase inhibitor  $\alpha$ 1-antitrypsin; the second pattern involved the immediate disruption of the epithelium, ciliary dyskinesia and ciliary stasis and was inhibited by extraction of chloroform, a recognised bacterial product (Sykes et al., 1987). A separate study found that elastase released by *Pseudomonas aeruginosa* caused epithelial disruption and when combined with human neutrophil elastase a marked cytotoxic effect including epithelial cell and basement membrane detachment as well as mitochondrial damage was seen (Amitani et al., 1991). The effect of other products of *Pseudomonas aeruginosa* have been studied including pyocyanin which slows ciliary beat frequency and alkaline protease which has not been found to have an effect (Amitani et al., 1991).

#### *Abnormal mucus composition*

The efficiency of the mucociliary transport system depends on the quantity and rheological characteristics of the airway mucus. Airway mucus has several important properties that contribute to its function: viscoelasticity (provided predominantly by the mucin glycoproteins); spinability; contact angle and surface tension. An in vitro study using a mucus depleted bovine trachea found that sputum from patients with bronchiectasis was transported 15% slower than the mean transportability rate of sputum from healthy human controls (Wills et al., 1995). Later studies in bronchiectasis focused on altering the osmolality of the mucus to influence the degree of hydration and thus its physical properties. In a study of 15 sputum samples, the mean(SEM) osmolality of the sputum was 240(9)mosM/kg. Addition of sodium chloride improved the transportability index of the sputum from  $26 \pm 3$  to  $58 \pm 5$ . Other



means of increasing the osmolality of the mucus were explored (including glucose, mannitol and urea) and were found to also enhance transportability (Wills et al., 1997). A later study manipulated the osmolality of 10 separate sputum samples with the addition of sodium chloride and observed that increasing the osmolality improved transportability by 41%. The investigators postulated that hyperosmolality of the airway surface liquid induces the release of mediators such as prostaglandin E<sub>2</sub>, histamine and leukotriene C<sub>4</sub> which are known to stimulate ciliary beat frequency hence improving ciliary beat frequency and mucus transportability (Shibuya et al., 2003). The influence of inhaled mannitol on sputum rheology and ciliary transportability has been explored particularly with the intention for therapeutic intervention. It has been demonstrated to reduce interfacial tension and spinability as well as increasing the wettability and hydration of airway mucus (Daviskas et al., 2005).

A deficient mucociliary transport system leaves the airways vulnerable to persistent exposure to potential pathogens. In bronchiectasis, the airways are frequently chronically infected with organisms.

#### *Airways colonisation*

Chronic infection of the airways in bronchiectasis has been recognised since the disease was first described although more detailed knowledge of the microbiology of the airways was limited until the early 20th century. Initial sputum studies were small such as Boggs in 1905 and Voigt in 1911 whose studies each involved sputum from only 5 cases of bronchiectasis, but it was observed that all were infected with *Haemophilus influenzae* (Boggs, 1905, Allison PR, 1943). Further studies remained limited and relied more on information from aspirated lung abscesses or sinuses until the advent of the

bronchoscope when it became possible to examine bronchial secretions that were minimally contaminated by oropharyngeal flora. In 1950, Benstead published his paper entitled “The Flora of One Hundred Bronchial Secretions” which included secretions from 30 patients with bronchiectasis. A variety of pathogens were isolated from these patients’ secretions including *Haemophilus influenzae* (n=12), *Streptococcus pneumoniae* (n=10),  $\alpha$ haemolytic *Streptococci* (n=9),  $\gamma$ haemolytic *Streptococci* (n=7), *Neisseria catarrhalis* (n=6),  $\beta$ haemolytic *Streptococci* (n=3), *Staphylococcus albis* (n=1), *Staphylococcus aureus* (n=1) and coliforms (n=1). It was also observed that there was no growth from the secretions of 8 patients with proven bronchiectasis (Benstead, 1950). A later study investigated the infecting pathogens in 108 patients with cylindrical bronchiectasis, using bronchial washings to isolate potential pathogens. Different bacterial species and types were identified in the washings, including *Streptococcus mitis*, *Streptococcus salivarius*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Mycoplasma*, although again, in a small number the washings were reported as sterile (Rytel et al., 1964). A later study in Hong Kong proposed that geographical variation may influence the infecting pathogens isolated in bronchiectasis (Pang et al., 1989). In their study of 23 patients, bacteria isolated included *Pseudomonas aeruginosa*, *Klebsiella ozaenae* and *Pseudomonas fluroescens* which at that time had been reported far less frequently (if indeed at all) in the predominantly European studies. Their conclusion has not been supported by later, larger studies. Microbiological studies over the past decade have however confirmed the early findings that the majority of patients with bronchiectasis are chronically infected or colonised with a variety of pathogens in their sputum even when clinically stable. A cohort study of 150 patients with bronchiectasis observed for a 12month

period found 66% to be chronically colonised (Pasteur et al., 2000) and similar rates were noted in a separate study of 77 patients of whom 64% were infected with pathogens in their sputum (Angrill et al., 2002). The most common organisms isolated in the latter study were *Haemophilus influenzae* (55%) and *Pseudomonas aeruginosa* (26%). More recently, a prospective study of 89 patients over a five year period found 79% to be chronically infected with bacterial pathogens in their sputum when clinically stable (King et al., 2007). On initial assessment the two most common infecting pathogens isolated from sputum samples were *Haemophilus influenzae* (47%) and *Pseudomonas aeruginosa* (12%). Other pathogens identified included *Moraxella catarrhalis* (8%), *Streptococcus pneumoniae* (7%), *Staphylococcus aureus* (4%), *Escherichia coli* (2%), *Mycobacterium avium* complex (2%) and no pathogens in 21%. Five years later, 74% continued to be chronically infected with 56% isolating the same organism in their sputum.

The potential clinical relevance of the predominant infecting pathogen has been explored in a limited number of studies, with the greatest emphasis on the two most common infecting pathogens, *Haemophilus influenzae* and *Pseudomonas aeruginosa*.

Studies initially reviewed the impact of isolation of *Haemophilus influenzae* from sputum, based on its greater incidence (and thus presumed importance) in the early microbiological studies, as eloquently described by Allison in 1943

“...the conclusion is inescapable that nonencapsulated *Haemophilus influenzae* is responsible for keeping the chronic inflammatory process smouldering in bronchiectatic individuals...” (Allison PR, 1943, Allison, 1943).

In 1966, the persistent presence of *Haemophilus influenzae* was observed to be associated with a reduction in total lung capacity in a study of 42 patients with cylindrical and saccular bronchiectasis of whom 32 were followed for at least 3 years (Cherniack and Carton, 1966). Patients with *Haemophilus influenzae* infection were later recognised to also have a poorer health related quality of life compared with those not chronically infected with pathogens in their sputum (Wilson et al., 1997b).

*Pseudomonas aeruginosa* colonisation is associated with more severe bronchiectasis. In an early study comparing 12 patients with a diagnosis of chronic bronchitis and *Pseudomonas aeruginosa* infection with 12 patients who had chronic bronchitis alone, computed tomography scans demonstrated that in the group infected with *Pseudomonas*, there was a greater presence and extent of bronchiectasis and a greater degree of bronchial wall thickening (Nagaki et al., 1992). These observations were echoed in a later study of 22 patients with chronic *Pseudomonas aeruginosa* infection with more extensive bronchiectasis, increased bronchial wall thickening and luminal dilatation as well as a greater area of reduced attenuation on CT chest scans than in patients not colonised with *Pseudomonas* (Miszkiel et al., 1997). *Pseudomonas aeruginosa* infection in a later study was also associated with poorer lung function compared with patients colonised by other organisms (Evans et al., 1996). A larger study of 100 patients of whom one third had chronic *Pseudomonas aeruginosa* infection similarly found that these patients had a lower FEV<sub>1</sub>/FVC ratio and also expectorated larger volumes of sputum than patients colonised with other organisms (Ho et al., 1998). The chronicity of *Pseudomonas* infection and potential impact on lung function was demonstrated in a study of 163 patients with bronchiectasis of whom 67 had never

been infected, 82 were intermittently infected and 14 were chronically infected with *Pseudomonas*, with the latter group having the lowest FEV<sub>1</sub> (Davies et al., 2006). However, it is questionable as to whether colonisation with *Pseudomonas* is a marker of disease severity rather than an entity responsible for decline in respiratory health (Evans et al., 1996). Patients with a history of chronic *Pseudomonas aeruginosa* infection have a worse health related quality of life than patients infected with *Haemophilus influenzae* or indeed other pathogens (Wilson et al., 1997b).

Specific studies of other pathogens that chronically infect the airways in bronchiectasis are limited. *Moraxella catarrhalis* is frequently isolated and although it has been demonstrated that the strain of the organism may vary over time, the type of strain has not been proven to correlate with exacerbations or antibiotic therapy (Klingman et al., 1995). Chronic *Staphylococcus aureus* infection is frequently associated with bronchiectasis affecting the upper lobes and in patients with allergic bronchopulmonary aspergillosis infection (Shah et al., 1999).

Non-tuberculous mycobacteria (NTM) may also be isolated and such infection is recognised as a cause as well as a complication (chronic airways infection) of bronchiectasis. Initially only a very few studies attempted to assess the extent of NTM infection in bronchiectasis, finding the incidence to be low (Bates, 1967, Wolinsky, 1979). In a later study of 91 patients with bronchiectasis followed for over 6 years, there was a 13% (n=12) incidence of positive Mycobacterial cultures, of which 3 were due to non-tuberculous Mycobacteria (Chan et al., 1992). More recently, a prospective study of 100 patients with bronchiectasis in 2005 found the prevalence of NTM to be 2%, but with only 1% requiring treatment (Wickremasinghe et al., 2005). A later study similarly

found a prevalence of 9% (7 out of 80 patients) with only 2.5% requiring treatment (Fowler et al., 2006). Little is known regarding the clinical impact of chronic infection with MAC and longitudinal clinical and radiological studies are needed.

Bacterial infection in the airways provokes a defensive inflammatory response. With chronic airway bacterial colonisation and invasion of the respiratory mucosa, a persistent, disproportionate inflammatory response occurs and there is progressive lung damage (Wilson et al., 1996).

#### *Airways Inflammation*

The role of neutrophilic inflammation in bronchiectasis is well recognised from findings of early sputum studies, imaging studies and studies of bronchial washings and mucosa (Stockley et al., 1984b, Currie et al., 1987c, Gaga et al., 1998).

An early study of uptake of radiolabelled polymorphonuclear leucocytes in 18 patients with severe, stable bronchiectasis demonstrated almost exclusive accumulation in bronchiectatic areas of the lungs in nearly 90% (n=16) within 24 hours (Currie et al., 1987c). A later study of bronchial mucosa observed significantly greater numbers of leukocytes, particularly neutrophils and macrophages in the airways of patients with stable bronchiectasis compared with healthy controls (Gaga et al., 1998). Endobronchial biopsies in a separate study had similar findings using monoclonal antibodies to stain neutrophils, macrophages and cells positive for TNF $\alpha$  with high densities of all observed in the lamina propria (Zheng et al., 2001). Early sputum studies found high quantities of the neutrophil protease elastase with strong correlation between elastase activity and sputum purulence (Stockley et al., 1984b). Bronchoalveolar lavage studies

have also found greater concentrations elastase and neutrophil degradation products such as myeloperoxidase in patients with bronchiectasis than in patients with other inflammatory lung conditions such as pneumonia and idiopathic pulmonary fibrosis (Schaaf et al., 2000).

#### *Neutrophil recruitment*

There is persistent polymorphonuclear leukocyte recruitment to the diseased airways in bronchiectasis (Stockley et al., 1988). Various chemoattractants are required for this migratory process, such as interleukin-8 (IL8), interleukin 1 $\beta$  (IL1 $\beta$ ), leukotriene B4 (LTB4), tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), transforming growth factor 1 $\beta$  (TGF1 $\beta$ ) and have been explored in the sputum of patients with bronchiectasis. IL8 and LTB4 have been found in high levels and with a strongly positive correlation with purulent sputum in bronchiectasis (Mikami et al., 1998). Bronchial mucosal biopsies have shown greater quantities of IL8 than controls (47cells/mm<sup>2</sup> compared with 15cells/mm<sup>2</sup> in healthy controls) (Gaga et al., 1998) and also TNF $\alpha$  (Zheng et al., 2001). TGF1 $\beta$  has been observed in higher concentration in the bronchial washings of patients with stable bronchiectasis compared with controls with a median(range) of 1,812.5(1226.4-4114.5)pg/litre compared with 1,342.4(940.3-2371.7)pg/litre. Dysregulation of such pro-inflammatory cytokines leading to their excessive release as indicated in the aforementioned studies, or improper countenance of their release by anti-inflammatory cytokines, leads to neutrophil recruitment and activation disproportionate to the offending pathogen with subsequent local airways damage.



### *Neutrophil activity*

Neutrophils recruited to the airways become activated, releasing proteases and free radicals as well as inducing cytokine release resulting in further and persistent stimulation of the inflammatory process. Airway secretions from bronchiectasis patients have been explored for a variety of such neutrophil products.

Neutrophil elastase is a serine proteinase produced during the early stages of neutrophil differentiation. It is released when the neutrophil becomes activated causing epithelial damage (Amitani et al., 1991), reducing ciliary beat frequency (Smallman et al., 1984), producing mucus gland hyperplasia (Snider et al., 1985) and stimulating mucus secretion (Sommerhoff et al., 1990). It damages immunoglobulins and opsonophagocytic receptors and promotes neutrophil influx by stimulating epithelial cells to produce chemoattractants (Tosi et al., 1990). High levels are found in the sputum of patients with bronchiectasis even when clinically stable (Tsang et al., 2000). Elastase content in sputum has been shown to positively correlate with increased 24hrsputum volume and number of lobes affected by bronchiectasis and in the same study had a negative correlation with FEV<sub>1</sub>percent predicted and FVC percent predicted (Tsang et al., 2000). Elastase activity has also been demonstrated to be higher with increasing sputum purulence (Stockley et al., 1984b, Lloberes et al., 1992).

Myeloperoxidase is a separate product of neutrophilic inflammation relevant to bronchiectasis. Initially named verdoperoxidase due to its intense green colour (Agner, 1941), it is a haem protein, abundantly expressed in neutrophils representing 5% of their total protein content (Klebanoff, 2005). It is released from cytoplasmic granules of neutrophils and catalyses the conversion of hydrogen peroxidise with chloride ions into



hypochlorous acid, a potent oxidant with cell damaging properties (Klebanoff, 1999). Myeloperoxidase also has proinflammatory properties independent of its enzymatic activity, activating CD11-b and CD18 on the outer membrane of neutrophils contributing to further neutrophilic recruitment (Lau et al., 2005). Excessive neutrophil accumulation can lead to inefficient clearance of apoptotic neutrophils by overwhelmed macrophages leading to secondary necrosis of neutrophils during which they release their lysosomal constituents with damaging effects to neighbouring host cells (Medan et al., 2002).

Other cells involved in the innate inflammatory process in the airways have been studied in patients with bronchiectasis, such as monocytes and mast cells. Higher spontaneous adherence of monocytes to fibronectin in the extracellular matrix in the airways of patients with bronchiectasis compared with healthy controls has been demonstrated, with increasing purulence associated with greater adherence (in healthy controls  $20\pm2\%$  adherence, patients with mucoid sputum  $40\pm8\%$  adherence and patients with purulent sputum  $65\pm5\%$  adherence) (Owen et al., 1992). Tryptase levels as a marker of mast cell activity have also been studied, with significantly higher levels detected in the bronchial washings of 36 patients with bronchiectasis compared with 14 healthy controls [ $4.7(1.4-20.1)\mu\text{mol/litre}$  versus  $2.0(0.1-3.55)\mu\text{mol/litre}$  respectively]. With increasing severity of bronchiectasis, there was noted to be a progressively higher concentration of tryptase: patients with mild disease had  $3.8(0.9-10.8)\mu\text{mol/litre}$ , moderate disease had  $4.3(3.0-12.6)\mu\text{mol/litre}$  and severe disease had  $9.6(1.2-20.1)\mu\text{mol/litre}$  (Sepper et al., 1998).

### *Bacterial infection and inflammation*

Inflammation in the airways is continuously stimulated by persistent bacterial colonisation of the airways. The bacterial density in the airways has been shown to correlate with the degree of airways inflammation. A study of 160 patients with chronic bronchitis that included 43 patients with idiopathic bronchiectasis observed that concentrations of the neutrophil chemoattractants IL8 and LTB4 and the neutrophilic degradation product myeloperoxidase in sputum increased with increasing bacterial density in the sputum (Hill et al., 2000). The concentration of myeloperoxidase(units/ml) increased from 0.3(0.1-0.5) in patients who were not chronically infected, to 0.5(0.2-0.7) with airway bacterial loads of  $10^5$ - $10^6$  colony forming units(cfu)/ml, to 0.5(0.3-1.2) with airway bacterial loads of  $10^6$ - $10^7$  cfu/ml, to 0.7(0.3-1.2) with airway bacterial loads of  $10^7$ - $10^8$  and with airway bacterial loads of  $>10^8$ , myeloperoxidase concentrations were 2.4(0.7-4.8). Bacterial species also influenced myeloperoxidase concentration, with patients infected with *Pseudomonas aeruginosa* having the greatest quantity of myeloperoxidase compared with those colonised with *Haemophilus influenzae* and *Moraxella catarrhalis* respectively. A later study of bronchial washings taken from 49 patients exclusively with bronchiectasis who were all clinically stable compared with 9 healthy controls echoed these findings, with a greater percentage of neutrophils [37(0-98)% versus 1(0-4)%], greater levels of elastase [90.5(8-2,930)µg/l versus 34(9-44)µg/l] greater levels of myeloperoxidase [9.1(0-376) u/l versus 0.3(0.1-1.4)u/l], higher levels of TNFα [4(0-186)pg/ml versus 0(0-7)pg/ml], higher levels of IL8 [195(0-5,520)pg/ml versus 3(0-31)pg/ml] and higher levels of IL6 [6(0-115)pg/ml versus 0(0-3)pg/ml] respectively (Angrill et al., 2001). With increasing

bacterial density in the airways of patients with bronchiectasis, there is an exaggerated inflammatory response with greater numbers of neutrophils and higher concentrations of neutrophil degradation products and other inflammatory markers.

### *Systemic inflammation*

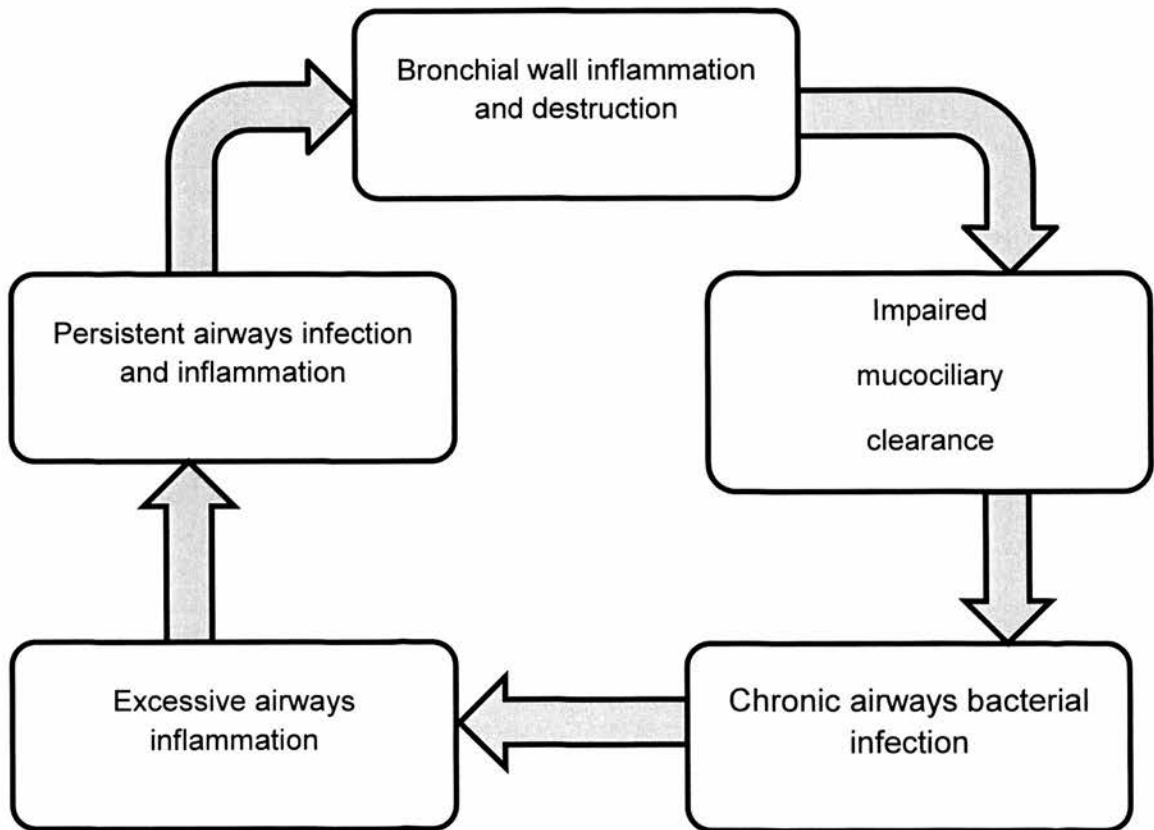
Studies of systemic inflammation in bronchiectasis are limited. Various markers of systemic inflammation have been investigated including serum markers and clinical features. Patients with bronchiectasis have been noted in one study to have a lower body weight and the same study also found that these patients had a lower serum albumin, but higher levels of serum antiproteases ( $\alpha$ 1 antitrypsin), serum globulins and leukocytes (Ip et al., 1991). A later study found that serum C-reactive protein was more sensitive than serum albumin in detecting a higher degree of airways inflammation as expressed by the concentration of neutrophil elastase in sputum (Lloberes et al., 1992). A further study found that peripheral leucocyte count, neutrophil number and the erythrocyte sedimentation rate all correlated with more extensive disease and poorer lung function in patients with bronchiectasis, but no relationship was found between these markers and sputum bacteriology (Wilson et al., 1998). In bronchiectasis, there is marked airways inflammation and further studies are needed to determine if there is a link with systemic inflammation.

### *The vicious cycle*

The impaired host defences in the damaged bronchiectatic airways, with persistent bacterial invasion and colonisation of the respiratory mucosa resulting in an inflammatory response that is continuous, exaggerated and poorly regulated has resulted in the hypothesis that a vicious cycle of perpetual infection, inflammation and destruction exists in the airways (Cole, 1986), Figure 5.

It is widely acknowledged that this vicious cycle of infection and inflammation is responsible for the persistent symptoms, frequent exacerbations and on-going airways damage in bronchiectasis. Acute infective exacerbations provoke intense episodes of inflammation with subsequent further disruption to the integrity of the airways and worsening of the vicious cycle in the airways.

Figure 5. The vicious cycle of infection and inflammation in the airways in bronchiectasis.



## **1.8 Current Management of Bronchiectasis**

The aims of management of bronchiectasis are to reduce symptoms (reduce cough frequency and severity, improve sputum volume and purulence, reduce breathlessness, chest pain and fatigue), reduce exacerbation frequency and severity, preserve lung function and improve health related quality of life. The impact of bronchiectasis on mortality is unclear but studies have explored factors that may predict mortality and these should be considered in the management of bronchiectasis.

There has been one long term follow-up study of risk factors for mortality in bronchiectasis (Loebinger et al., 2009b). Over a 13 year period, 91 patients with bronchiectasis were studied and increased age, a history of *Pseudomonas aeruginosa* colonisation, reduced total lung capacity, reduced residual volume to total lung capacity ratio, reduced transfer factor coefficient and a poorer score in the activities section of the St George's Respiratory Questionnaire (SGRQ) were all identified as risk factors for increased mortality. Acute exacerbations of bronchiectasis requiring inpatient management may also be a risk factor for mortality as Roberts et al observed that 21% of patients had died within 12 months of an admission with an exacerbation of bronchiectasis to a New Zealand hospital in 2002 (Roberts et al., 2011). A separate study explored predictors for mortality in 43 patients who had been hospitalised with an acute exacerbation of bronchiectasis and found a hospital mortality of 9% and a one year mortality of 30% (Finklea et al., 2010). Factors associated with increased mortality included male sex, reduced FEV<sub>1</sub> %predicted, smoking history, use of systemic steroids, elevated serum creatinine concentration and a need for mechanical ventilation. Potentially treatable risk factors or higher risk patients requiring more aggressive

management that should be targeted therefore include those colonised with *Pseudomonas aeruginosa*, those with reduced total lung capacity and gas transfer factor on pulmonary function testing and patients with exacerbations requiring hospitalisation.

The approach to management is multifaceted. Current strategies include medical management such as patient education, advice on airway clearance and management of exacerbations as well as consideration of long term treatment strategies such as anti-inflammatory and or antimicrobial therapies. Surgical intervention, once the only management available for patients with bronchiectasis also continues to offer a potential therapeutic option.

#### *Patient Education*

Patients should understand the diagnosis of bronchiectasis, its natural history and implications particularly the importance of limiting further damage to their airways.

Smoking is a major insult to the airways. Inhalation of tobacco causes mucosal gland hypertrophy (Megahed et al., 1967), mucus hypersecretion and the recruitment of inflammatory cells into the lungs with the uncontrolled release of toxic free radicals. Such activity is destructive to the integrity of the already damaged airway walls. Furthermore, chronic smoking has been demonstrated to independently induce polymorphic ultrastructural axonemal abnormalities in bronchoepithelium cilia which may impair tracheobronchial clearance of secretions (Verra et al., 1995). Smoking has also been found to be a risk factor for increased mortality in bronchiectasis (Finklea et al., 2010). Advice on smoking cessation should be given to all patients with

bronchiectasis both to limit any further damage to the airways, reduce exacerbation frequency and to improve mortality.

Limiting infective insults to the airways through early recognition and treatment of exacerbations is important and patients should be educated to have an awareness of any deterioration in their symptoms and know to seek help. Prevention of pneumonia and viral insults may be limited by receiving the annual seasonal influenza vaccination and the pneumococcal vaccine. Studies are however, limited. Furumoto et al (Furumoto et al., 2008) studied 167 patients with chronic lung disease including bronchiectasis and found that patients receiving both the 23-valent pneumococcal vaccine and the influenza vaccine had significantly fewer acute infective exacerbations over a two year period than patients receiving the influenza vaccine alone. There was however, no significant difference found in episodes of pneumonia.

#### *Airway Clearance*

The normal mucociliary clearance mechanism in bronchiectatic airways is impaired, mucus stagnates and the airway walls become further inflamed and damaged. Effective mobilisation and clearance of secretions may be promoted with chest physiotherapy. Other techniques for airway clearance have been proposed either as independent treatments or more frequently as adjunctive measures to physiotherapy including airways humidification, nebulised isotonic and hypertonic saline, nebulised  $\beta_2$  agonists, inhaled mannitol, nebulised recombinant deoxyribonuclease and mucolytics.



## *Physiotherapy*

Breathing and exercise were proposed as important adjuncts to medical and surgical treatments by Macmahon in 1915 (Macmahon, 1915). Over the past century, different techniques and devices for chest physiotherapy have evolved and are used in the ongoing management of bronchiectasis including postural drainage, the active cycle of breathing techniques (ACBT), inspiratory muscle training, positive expiratory pressure (PEP) and oscillatory positive expiratory pressure devices. A UK survey of routine practice in 2002 found the most commonly practised techniques to be ACBT and postural drainage (O'Neill et al., 2002).

Postural drainage was first suggested by Quincke in 1898 and essentially describes gravity assisted drainage of secretions. Quincke postulated that postural drainage had 4 main mechanisms of action: firstly, the mechanical influence of gravitation to drain the mucus; secondly, the subsequent movement of mucus through the airways provided a stimulus for the cough reflex; thirdly, previously airless parts of the lung could then expand and finally the pressure of the intra-abdominal organs on the diaphragm would also facilitate expectoration (Quincke, 1898). Ewart in 1901 reported two case studies and advised the technique should be used continuously for bronchiectasis and “chronic bronchial affections”. He conceded that only if poorly tolerated then it could be practised intermittently, for at least 3 hours per day (Ewart, 1901). Postural drainage remained the technique of choice throughout the following five decades with further studies including Nelson in 1934 who proposed that a position of fifteen degrees of the Trendelenburg position would assist with middle lobe drainage and at twenty degrees of the Trendelenburg position the lower lobes would drain (Nelson, 1934). The technique

does not require any equipment and given adequate space can be practised unassisted anywhere. Disadvantages include possible promotion of gastro-oesophageal reflux and breathlessness. Chronic or increased gastro-oesophageal reflux may be a risk factor for bronchiectasis and should the technique causes occurrence or worsening of such symptoms then its practice would not be recommended. Dyspnoea with postural drainage may also limit compliance (Cecins et al., 1999). In the 1960s, alternative physiotherapy strategies to postural drainage began to emerge including different exercise techniques and the use of specialised devices. The active cycle of breathing techniques originated in New Zealand and involves a pattern of tidal breathing at the patient's own rate followed by thoracic expansion exercises then forced expiration (huffs) and a cough. Huffing generates both a shearing force within the airways and an oscillatory movement of the airway wall itself helping to reduce mucus viscosity and mobilise peripheral secretions to the more proximal and larger airways where the final cough will clear them. Devices such as the Flutter create a positive expiratory pressure and an oscillatory vibration of air within the airways, loosening and mobilising secretions to the larger proximal airways for clearance with cough. Several short term small studies have compared the various techniques and to date, no single method has been found to be superior.

Postural drainage was formally studied by Mazzocco et al in 1985 in thirteen outpatients with bronchiectasis. A single ten minute session of postural drainage (at ten degrees of the Trendelenburg position) was compared with a ten minute session of postural drainage and chest percussion (Mazzocco et al., 1985). No difference was seen in FEV<sub>1</sub>, FVC, peak expiratory flow, oxygen saturations or sputum volume. Following both

techniques, the authors observed that between 2-50% of the patients' total daily sputum volume was expectorated. Cecins later revisited the benefits of postural drainage with the active cycle of breathing technique (Cecins et al., 1999). In their study, nineteen patients each underwent a single session of ACBT alone; ACBT with a twenty degree tilt; ACBT with a one hundred and eighty degree tilt. Each session was separated by a twenty four hour washout period. No session was superior, with no significant difference seen in FEV<sub>1</sub>, oxygen saturations or sputum weight measured immediately after each session. However, using a visual analogue scale, a significant number of patients reported increased dyspnoea following the session with a twenty degree tilt and only one patient reported a preference for the twenty degree tilt session.

The longest study to date of chest physiotherapy in bronchiectasis compared four weeks of ACBT with four weeks of twice daily usage of the flutter device in seventeen patients with bronchiectasis and found no significant difference between the techniques in sputum weight, dyspnoea score using the BORG scale or in health related quality of life using the Chronic Respiratory Disease Questionnaire. A statistical improvement was seen in FEV<sub>1</sub> but this was not thought to be clinically significant (Thompson et al., 2002). Other studies have involved outcome assessment following single physiotherapy sessions with different techniques and have found no difference in spirometry, oxygen saturation or sputum weight (Patterson et al., 2005, Eaton et al., 2007, Patterson et al., 2004).

Acceptability of a technique is recognised as critical for regular compliance. As no single technique has been proven to be superior, the prescribed physiotherapy regimen should be individualised and guided by patient preference.

### *Pulmonary rehabilitation*

Pulmonary rehabilitation involves a combination of exercise training sessions (such as treadmill walking, stair climbing, cycling) and multidisciplinary education delivered on a regular basis over a 6 to 8 week period. Its efficacy in COPD is well established (Nici et al., 2006). A randomised controlled trial of 32 patients with bronchiectasis investigated the effect of pulmonary rehabilitation and inspiratory muscle training (IMT) with pulmonary rehabilitation and an IMT sham with a control group. At 8 weeks an improvement was seen in both groups (although there was no significant difference between the groups) but at 12 weeks the improvement in exercise capacity had only been maintained in the IMT group. The study did not find the addition of IMT to the pulmonary rehabilitation programme provided any further significant benefit (Newall et al., 2005). More recently, a retrospective study of 95 patients with bronchiectasis demonstrated significant improvements in both exercise capacity (assessed using the 6 minute walking distance test) and in health related quality of life (assessed using the Chronic Respiratory Disease Questionnaire). 37 of these patients were followed up 12 months after the pulmonary rehabilitation programme and still had a better total exercise capacity than at entry to the pulmonary rehabilitation programme (Ong et al., 2011).

### *Airways humidification*

Adequate hydration of the periciliary layer is necessary for optimal ciliary transport capacity (Litt, 1970) and cough efficiency is known to improve with greater depth of the periciliary fluid layer and reduced mucus viscosity (Scherer, 1981). Bronchiectatic airways have a damaged epithelial layer and periciliary hydration may be suboptimal. The rationale for humidification of the airways is that it may increase periciliary fluid

depth and reduce mucus viscosity thus enhancing ciliary movement and mucus flow in the airways. Evidence is, however, limited. One study has attempted to quantify the effect of thirty minutes of cold water jet nebulisation to the airways as an adjunct to postural drainage (with a twenty degree tilt) and huffing (Conway et al., 1992). Seven patients with bronchiectasis underwent a single session of such humidification and physiotherapy and one week later a session of physiotherapy alone. Sputum clearance was assessed using labelled Technetium with gamma camera imaging. Sputum weight and FEV<sub>1</sub> also observed. A significant increase in sputum weight (median increase of six grams) and sputum clearance (median increase of 8.7%) was observed following both humidification and physiotherapy compared with physiotherapy alone. Similar improved mucociliary clearance was seen following seven days of domiciliary humidification (daily three hours' inhalation of saturated air at 37°C) in ten patients with bronchiectasis with the area under the tracheobronchial retention curve reducing from 319±50%/hr to 271±46%/hr (Hasani et al., 2008). The largest clinical study to date, although not exclusively in patients with bronchiectasis, was a randomised controlled trial of 108 patients with COPD or bronchiectasis which found that following twelve months' domiciliary humidification treatment patients had fewer and shorter exacerbations with a greater length of time to their first exacerbation compared with patients receiving routine care (Rea et al., 2010). No other formal studies of airways humidification in bronchiectasis have been undertaken and although it may confer some benefits, the current evidence is insufficient to recommend it for routine use, particularly given the potential risk of infection from the delivery systems with opportunistic organisms especially *Mycobacteria*.

### *Nebulised saline and $\beta_2$ agonists*

Efficiency of the mucociliary transport mechanism is intrinsically related to the constituents and properties of the mucus. The rationale for nebulised saline as an adjunct to physiotherapy is that it may alter the concentration of the main constituent of mucus (i.e. water) thus improving transportability (Shibuya et al., 2003). Clinical studies are, however, limited. In 1988 Sutton investigated whether isotonic (0.9% saline) or nebulised  $\beta_2$  agonists were useful adjuncts to ACBT physiotherapy. Their study involved only 8 patients undergoing single treatment sessions but did find that with both nebulised adjunctive treatments sputum yield was greater than with ACBT alone. In 2005 Kellett et al allocated 24 patients with bronchiectasis to receive four separate sessions of ACBT physiotherapy with three of the sessions involving adjunctive treatment with a nebulised  $\beta_2$  agonist and either nebulised 0.9% saline or nebulised 7% saline (Kellett et al., 2005). With nebulised 7% saline, the weight of sputum expectorated was greater than with 0.9% saline and there was a notable linear trend towards reduced sputum viscosity with the hypertonic saline. However patients reported greater ease in expectoration with 7% saline. These studies provide sufficient evidence to support a larger, longer-term study into the role of nebulised saline as an adjunctive therapy to regular physiotherapy but at present such nebulised treatments are not routinely used in clinical practice.

### *Inhaled hyperosmolar agents*

Mannitol is a naturally occurring sugar alcohol that acts as a non-ionic osmotic agent when inhaled as a dry powder into the airways. The mannitol increases the osmolarity within the airway lumen causing water to leave the epithelial cells with their subsequent

shrinkage causing several cell volume regulatory actions to occur, including intracellular influx of calcium (Korngreen and Priel, 1994). Increased intracellular calcium increases ciliary activity and will thus aid the mucociliary transport mechanism (Eveloff and Warnock, 1987). Hyperosmolarity within the airways is also thought to trigger mast cell degranulation with release of histamine which may also contribute to increased ciliary beat frequency (Silber et al., 1988).

Mannitol has been demonstrated clinically to improve mucociliary clearance in bronchiectasis. An open label study using a radioaerosol technique to assess mucus clearance in 11 patients with bronchiectasis observed that  $34.0 \pm 5.0\%$  mucus clearance was achieved 75minutes following inhalation of a mean(standard deviation) dose of  $320 \pm 81\text{mg}$  of dry powder mannitol compared with  $17.4 \pm 3.8\%$  clearance with control (Daviskas et al., 1999). A later study using the same technique in 8 patients found that a single inhaled dose of 400mg of dry powder mannitol reduced the extent of mucus retained within the lung 24hours after inhalation suggesting that mannitol has useful long-acting effects in addition to its acute impact (Daviskas et al., 2001). Clinically, an open label study of 12 days treatment with daily 400mg inhaled dry powdered mannitol improved small airways function (using midexpiratory flow  $\text{FEF}_{25-75}$ ), improved cough transportability and had a beneficial effect on health related quality of life (Daviskas et al., 2005). A multicentre clinical study has recently been completed and the outcomes are currently awaited. At present, there is insufficient evidence to support its routine use.



### *Recombinant human deoxyribonuclease 1*

Purulent airway secretions contain excessive quantities of deoxyribonucleic acid (DNA) released by persistent and uncontrolled neutrophilic activity in the chronically inflamed and infected airways. DNA is an extremely viscous polymer and a major contributor to the tenacity of purulent secretions. Inhalation of recombinant human deoxyribonuclease (rhDNase) should cleave the DNA, reducing sputum viscosity, improving expectoration, airway clearance and lung function. However, its routine use in bronchiectasis is not advised after a randomised double blind placebo controlled trial of rhDNase in 349 patients for 24 weeks found that patients randomised to twice daily treatment with rhDNase had a higher rate of exacerbations, increased use of antibiotics and a greater rate of hospitalisations as well as a significant reduction in FEV<sub>1</sub> (O'Donnell et al., 1998).

### *Mucolytics*

There are very few studies exploring the efficacy of other mucolytics in bronchiectasis. Erdosteine has been demonstrated to significantly improve mucociliary transport velocity in ex-smokers with chronic bronchitis and hypersecretion (Olivieri et al., 1991). A more recent study assessed the efficacy of oral erdosteine in 30 patients in a prospective parallel open label pilot study of twice daily erdosteine and physiotherapy versus physiotherapy alone. Although both groups demonstrated improvements in the 6 minute walk test and visual analogue scores of cough, dyspnoea, sputum volume, colour and purulence, the erdosteine group had a significant improvement in FEV<sub>1</sub> and FVC (Crisafulli et al., 2007). There is currently insufficient evidence to support the routine use of mucolytics in the management of stable bronchiectasis.



### *Anti-inflammatory strategies*

Limiting the perpetual inflammation in bronchiectasis may reduce the persistent damage to the airway walls and disrupt the vicious cycle of inflammation and infection.

Anti-inflammatory strategies include inhaled corticosteroid therapy and more recently the immunomodulatory effects of macrolide antibiotic therapy have been proposed as potential treatment strategies.

### *Inhaled corticosteroids*

The precise mechanism of action of inhaled corticosteroids in bronchiectasis is not known. However, both sputum studies and studies of bronchial epithelium have demonstrated a reduction in inflammatory cells with inhaled corticosteroid use. Patients with bronchiectasis have high total leucocyte counts within the bronchial epithelium and evidence of mucus gland hypertrophy (Gaga et al., 1998). In a study of endobronchial biopsies in 12 patients with bronchiectasis, 6 of whom were on regular inhaled corticosteroid treatment, there were significantly fewer CD3+ and CD4+ T lymphocytes in the patients receiving inhaled corticosteroids (CD3+ 197cells/mm<sup>2</sup> versus 369cells/mm<sup>2</sup> respectively and CD4+ 82cells/mm<sup>2</sup> versus 190cells/mm<sup>2</sup> respectively). Additionally, there were fewer cells positive on staining for interleukin-8 (30cells/mm<sup>2</sup> versus 60cells/mm<sup>2</sup>) (Gaga et al., 1998). A four week double blind randomised controlled trial of twice daily 500µg fluticasone versus placebo in 24 patients with bronchiectasis found a significant reduction in sputum leucocyte density, interleukin (IL)-8, IL-1β and leukotriene B<sub>4</sub> in the treatment group (Tsang et al., 1998a). It is therefore postulated that inhaled corticosteroids may exhibit an effect in bronchiectatic

airways by a reduction in inflammatory cell recruitment with subsequently less proinflammatory mediator release and activity. There have been only a few studies of the clinical benefits of inhaled corticosteroids in bronchiectasis. A double blind placebo controlled crossover study of 20 patients explored the effect of 750µg of beclometasone administered twice daily for six weeks on sputum production, lung function and respiratory symptoms. There was an 18% reduction in daily sputum production, an improvement in cough on symptom scoring and a statistically significant increase in peak expiratory flow rate and FEV<sub>1</sub> (although the authors queried the clinical relevance of these latter improvements) with regular inhaled corticosteroid use (Elborn et al., 1992). A larger and longer term study of 500µg fluticasone twice daily versus placebo in 86 patients over a 12 month period found that with treatment, 24hour sputum volume reduced but there was no difference in sputum purulence, lung function or exacerbation frequency (Tsang et al., 2005a). A further study explored the effect of inhaled corticosteroid dosage in a six month randomised controlled trial of 93 patients who received either 250µg fluticasone twice daily or 500µg fluticasone twice daily or placebo (Martinez-Garcia et al., 2006). Patients receiving 500µg fluticasone twice daily reported less cough, had less daily sputum [-9.7(12.8)mls per day], a reduction in their dyspnoea and had an improved score on the health related quality of life questionnaire the SGRQ [improved from a score of 45.5(14.2) to 40.5(13.9) after 6 months treatment]. No significant changes were seen in any group in pulmonary function or exacerbation frequency. The evidence to support long term use of inhaled corticosteroids in bronchiectasis is limited and their use must be balanced against the risk of adverse effects. A study of 50 patients with bronchiectasis of whom 33 were receiving regular inhaled corticosteroids found that 48.5% had adrenal suppression and a poorer health

related quality of life score when investigated with a short synacthen test and completion of the SGRQ compared with only 23.5% of those not regularly receiving inhaled corticosteroids (Holme et al., 2008). However, patients may warrant a trial of therapy particularly those with excessive sputum volume.

### *Macrolide therapy*

Macrolides were originally discovered as antibacterials that act through the inhibition of RNA dependent protein synthesis by reversibly binding to the 50S ribosomal unit of a susceptible microorganism. Interest in potential immunomodulatory properties of the 14-member (erythromycin and clarithromycin) and 15-member (azithromycin) macrolides occurred in the 1960s and clinically these effects were first reported in asthma by Spector et al in 1974 (Spector and Farr, 1974). Since then, their role has been studied in other chronic inflammatory pulmonary diseases such as diffuse panbronchiolitis, COPD and cystic fibrosis but to date there have only been four studies in adults with bronchiectasis. The mechanism of action is thought to be immunomodulatory rather than immunosuppressive and includes: inhibition of inflammatory cell chemotaxis through suppression of cytokine production such as IL-8, IL-6 and intracellular adhesion molecule (ICAM)-1 (Takizawa et al., 1997, Kusano et al., 1995); impairment of superoxide generation by activated neutrophils (Kadir et al., 2000) as well as inhibition of mucus hypersecretion (Goswami et al., 1990, Tamaoki et al., 1995).

The first clinical study of macrolide use in bronchiectasis was an 8 week randomised double-blind placebo controlled trial of erythromycin 500mg twice daily versus placebo in 21 patients. A significant improvement in FEV<sub>1</sub> and FVC was seen with a significant

reduction in 24hrsputum volume from a baseline of mean (95%confidence interval) 33.7(23-49.3)ml to 23.8(15.7-36.1)ml after 8 weeks' treatment. No changes were seen in sputum bacterial density or perhaps somewhat surprisingly in the inflammatory mediators IL-8, IL-1 $\alpha$ , TNF- $\alpha$  and leukotriene-B4. Azithromycin 250mg was used thrice weekly for four months in 39 patients with bronchiectasis in a study in 2004 with significant improvements seen in exacerbation frequency, use of intravenous antibiotic therapy, symptoms and gas transfer diffusing factor coefficient (Davies and Wilson, 2004). An open label randomised crossover trial of twice weekly 500mg azithromycin treatment in 11 patients found significant reductions in sputum volume and exacerbation frequency with treatment (Cymbala et al., 2005). The most recent study was an uncontrolled study of once daily 250mg erythromycin given for 12 months in 24 patients with bronchiectasis (Serisier and Martin, 2011). In comparison with the 12 months preceding entry into the study the median number of infective exacerbations halved [median(range) 2(0-8)exacerbations compared with 4(2-11) exacerbations respectively,  $P<0.0001$ ] and the number of days of antibiotic use per year also significantly reduced [median(range) 21(0-78)days compared with 44(15-138)days respectively,  $P<0.0001$ ].

The regular long term use of macrolides is associated with potential adverse effects including perhaps most importantly macrolide resistance. The risk of macrolide resistance in nontuberculous mycobacteria that may be more prevalent in patients with bronchiectasis needs explored. Other adverse effects include gastrointestinal upset (diarrhoea occurred in 25% of Cymbala's study population with treatment), derangement of liver function tests (Davies and Wilson, 2004) and rash (Tsang et al.,

1999b, Davies and Wilson, 2004) and potential middle ear problems with balance difficulties and deafness. However, both their potential immunomodulatory and antibacterial effect are intriguing and potentially offer a long term therapeutic option in bronchiectasis, although further randomised controlled studies are needed.

### *Antimicrobial strategies*

The rationale for prescribing long term antibiotics (oral or nebulised) in clinically stable bronchiectasis is to reduce the bacterial burden in the airways therefore limiting inflammation and promoting healing of the bronchial tree. Disrupting the vicious cycle of infection and inflammation should reduce daily symptoms and exacerbation frequency. The evidence for long term antibiotics is, however, limited. Studies have been conducted using both oral antibiotics and nebulised antibiotics, but the study designs have varied, used different criterion for selecting patients and different durations and doses of antibiotics with various outcome measures to assess response making comparisons between regimens difficult subsequently limiting clinical application. National guidelines published by the British Thoracic Society in 2010 recommended that patients suffering three or more exacerbations per year or those with fewer exacerbations but significant morbidity should be considered for long term antibiotics (Pasteur et al., 2010).

The first randomised controlled trial of long term oral antibiotic therapy was designed to study the effect of prolonged antibiotic therapy in severe cases of bronchiectasis characterised by abundant mucopurulent or purulent sputum. It was conducted in 112 patients from seven different UK centres over 12 months by the MRC in 1957 and compared 2g of tetracycline daily with 2g of penicillin daily or 2g of lactose daily. It

found that 2g of oral tetracycline per day improved sputum purulence and reduced sputum volume significantly (from 76.7ml to 49ml per day) as well as reducing the number of absent days from work due to respiratory ill health (118 days of absenteeism compared with 381 days in the penicillin group and 446 days in the lactose group) (1957). Tetracycline and penicillin continued to be studied as a potential long term oral antibiotics in studies conducted over the following few decades. Cherniack et al in 1959 found that with 2g tetracycline per day used for at least 3 months, there were fewer exacerbations and exacerbations that occurred were of shorter duration compared with placebo (Cherniack et al., 1959). In 1960, a randomised controlled trial of tetracycline 2g daily compared with penicillin 1g daily, compared with an oleandomycin/penicillin combination and placebo in 89 patients focussed on the impact of the treatments on sputum bacteriology. Tetracycline was effective in reducing infection with *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* but there was increased infection with *Pseudomonas aeruginosa*. Penicillin was effective in reducing *Streptococcus pneumoniae* infection but not *Klebsiella* species and the oleandomycin/penicillin combination was found to be effective in reducing infection with *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, but not *Proteus* species (Dowling et al., 1960). The effect of antibiotic therapy on sputum inflammatory indices was explored by Stockley in 1984, although the amoxicillin administered to the 15 patients in the open label study was for 14 days only. However, in the 67% patients who clinically responded with macroscopic clearing of sputum purulence and eradication of the sputum pathogen, there was a significant reduction in sputum elastase and albumin leakage (Stockley et al., 1984a). Later studies explored the effect of long term antibiotics on other clinical endpoints such as lung

function and found minimal improvements (Hill and Stockley, 1986). Three decades on, another randomised controlled trial was conducted to explore the impact of high dose daily oral amoxicillin (3g BD) over 8 months in 32 patients. It found that amoxicillin treatment led to clinical improvement, reduction in 24 hour sputum volume and purulence as well as fewer absent days from work, with the treatment well tolerated (Currie et al., 1990). Tolerability of long term oral antibiotics is a clear factor that may affect compliance and was recognised early on in antibiotic studies. Sobel et al assessed the adverse effects with long term usage of oral tetracycline, oral penicillin and oral olandeomycin/penicillin combination preparations (Sobel et al., 1962). Tetracycline was associated with reported diarrhoea in 27% and with both penicillin and olandeomycin/penicillin combinations upper gastrointestinal symptoms occurred (reported in 27% and 20% respectively). Aerosolised antibiotics began to be formally studied as potential means of delivering long term antibacterial therapy to the diseased airways in the 1980s. With direct delivery to the lungs, systemic absorption could be anticipated to be minimal with a lower risk of such adverse systemic effects.

Nebulised antibiotics offer a targeted therapy with limited systemic side effects, but are expensive and may be less well tolerated due to bronchospasm- even with adjunctive treatment with a  $\beta_2$  agonist. An open label study in 1985 assessed the impact of 4 months treatment with nebulised amoxicillin in six patients who had failed to respond to oral amoxicillin (Stockley et al., 1985). The randomised controlled trials of long term nebulised antibiotics began to be conducted in the late 1990s in patients chronically colonised with *Pseudomonas aeruginosa*. Orriols et al compared the impact of nebulised ceftazidime and nebulised tobramycin with placebo over 12 months in 15



patients and found that with nebulised antibiotic therapy there were fewer and shorter hospital admissions for exacerbations and no increase in antibiotic resistance (Orriols et al., 1999). Two larger randomised controlled trials followed comparing twice daily nebulised tobramycin with placebo, one cyclically (4 weeks treatment, 2 weeks off) and the other daily for 6 months. Both found a reduction in bacterial density in the sputum but there was a small increased incidence of bronchospasm in the treatment arms (Barker et al., 2000, Drobic et al., 2005). Further support for the role of nebulised tobramycin in patients colonised with *Pseudomonas aeruginosa* was demonstrated in an open label study in 2005 of 41 patients who received three cycles each of fourteen days of twice daily 300mg tobramycin separated by fourteen days with no treatment. Patients reported an improved health related quality of life and 22% achieved complete eradication of *Pseudomonas aeruginosa* from their sputum. However, 5% developed resistance to tobramycin and 22% stopped treatment early due to adverse effects (Scheinberg and Shore, 2005).

Further studies are needed to define who would benefit from long term antibiotics and the optimum mode and type of treatment.

### *Eradication*

There have been no studies in non-cystic fibrosis bronchiectasis evaluating whether a targeted antibiotic regime should be commenced following first isolation of organisms such as *Pseudomonas aeruginosa* and Methicillin resistant *Staphylococcus aureus* from the airways in otherwise clinically stable disease. The rationale for attempting bacterial clearance is founded upon these organisms infecting the airways of patients with more severe bronchiectasis with a poorer prognosis (Ho et al., 1998, Loebinger et al., 2009a,



Wilson et al., 1997b). Such an approach is used in cystic fibrosis bronchiectasis, but its effect and long term impact in non-cystic fibrosis bronchiectasis is unknown. The current UK national guidelines do however advise that eradication may be attempted as a pragmatic approach to management of the condition, although this advice is based on consensus expert opinion only and long term, randomised controlled studies are needed (Pasteur et al., 2010).

### *Surgery*

Prior to the introduction of antibiotic therapy, the most effective management of bronchiectasis was surgery.

*“The only means of “curing” bronchiectasis in the sense of causing the disappearance of the dilated bronchi is by surgical removal....”* (Warner, 1932).

Currently, surgical management is reserved for specific indications including major haemoptysis or localised disease causing significant morbidity refractory to medical management. Surgery has traditionally been performed via thoracotomy and reported outcomes from retrospective case reviews are good. An analysis of 92 patients who underwent thoracotomy between 2002-2007 including ten pneumonectomies, 36 lobectomies, 2 bi-lobectomies and segmentectomies and 12 segmentectomies) had a morbidity rate of 16% and mortality of 1%. With a mean follow-up of 15.3 months in 75 patients, 84% were asymptomatic and 10.7% had had a significant clinical improvement (Gursoy et al., 2010). A larger retrospective analysis of 277 patients undergoing thoracotomy (42.2% a unilateral lobectomy) for bronchiectasis had a mortality of 0.7% and morbidity of 15.8% with 68.5% of patients being symptom free

over a mean follow up of four and a half years (Bagheri et al., 2010). Risk factors affecting surgical outcome were explored in a retrospective analysis of 143 patients and included a previous history of tuberculosis infection, an FEV<sub>1</sub> of less than 60% predicted, incomplete resection and patients who had not undergone a preoperative bronchoscopy (Eren et al., 2007). More recently, outcomes from video assisted thoracoscopic surgery have been compared with thoracotomy. A study of 52 patients undergoing video assisted thoracoscopic surgery (VATS) compared with 52 undergoing thoracotomy found that the VATS group had a shorter length of stay post-operatively, had fewer post-operative complications and 94% achieved complete recovery compared to an 88% complete recovery in the thoracotomy (Zhang et al., 2011). In current practice, referral for surgery is not common and to date there have been no randomised controlled trials of surgical and nonsurgical management of bronchiectasis.

#### *Management of exacerbations*

Exacerbations of bronchiectasis requiring antibiotic therapy are typically defined as a persistent (more than 24 hours) deterioration in respiratory symptoms (increased cough, increased sputum volume, increased sputum purulence, breathlessness or wheeze) with or without systemic disturbance. They are responsible for both significant patient morbidity and socioeconomic cost as patients frequently utilise both primary and secondary health care resources. UK hospital data for 2007-2008 identified bronchiectasis as the primary diagnosis for 1 in 1800 inpatient admissions. In the United States between 1993 to 2006, the average annual age-adjusted hospitalisation rate was 16.5 per 100 000 of the population (Seitz et al., 2010). Kelly et al observed 410 patients for 6 months during which time 30 patients required a hospital admission, a further 12

required domiciliary intravenous antibiotic therapy and 77 received oral antibiotic therapy (Kelly et al., 2003). The associated days of absence from work have a wider societal impact.

More frequent severe exacerbations have been shown to be an independent factor for decline in lung function. In a study of 76 patients over 24 months the mean annual rate of severe exacerbations (those requiring treatment with systemic corticosteroids, or with respiratory failure or those requiring hospitalisation) was 1.5(range 0-8) (Martinez-Garcia et al., 2007). There are no randomised placebo controlled trials evaluating the efficacy of antibiotic treatment of infective exacerbations although studies have shown that treatment can improve patients' health related quality of life. A study of 18 patients requiring intravenous antibiotics for an infective exacerbation had a significant improvement in their quality of life score (improvements were seen in dyspnoea, emotional and mastery subsections) following completion of treatment (Courtney et al., 2008). Despite such morbidity and the cost of exacerbations to the patient and society, there is little evidence to guide selection of antibiotic(s), mode of delivery, duration of treatment or indeed the role of adjunctive therapies.

#### *Antibiotic therapy*

The first studies of antibiotic treatment for exacerbations were for exacerbations that had not responded to an initial course of antibiotics. Douglas et al randomised 131 patients who had not responded to penicillin (1 or 2 megaunits, administered intramuscularly for between 5 to 14 days) to receive either chloramphenicol (2 grammes per day, orally) or oxytetracycline (2 grammes per day, orally) for between 5 to 7 days. 78% of those treated with chloramphenicol developed mucoid sputum compared with

38% in the oxytetracycline group (Douglas et al., 1957). Further studies suggested that higher doses of antibiotics and longer treatment duration may have a greater impact. A randomised controlled trial of 14 days of penicillin and streptomycin compared with cephaloridine (2g daily or 4g daily or 6g daily) in 197 patients who had failed to respond to an initial course of antibiotics found that 6g of cephaloridine achieved conversion to mucoid sputum in 56% which was superior to penicillin and streptomycin which achieved mucoid sputum in only 34%. Subsequent to this study, Pines et al conducted a further randomised controlled trial comparing 4 separate antibiotic regimens in patients known to be chronically colonised with *Pseudomonas aeruginosa* with exacerbations and found greatest success with combinations of intramuscular or intravenous antibiotics and inhaled antibiotics administered for a 7 to 14 day period (Pines et al., 1970). These findings proposed that longer duration of antibiotic therapy, dual therapy and both local and systemic treatment may be a more effective approach to management of exacerbations. Later studies have ranged in duration of treatment from 7 to 14 days, but there have been no randomised controlled trials comparing treatment efficacy between 7 and 14 days. Mode of delivery of antibiotic was explored in 1994 in a study of 27 patients randomised to either intravenous antibiotics alone or intravenous and inhaled antibiotics for seven days. The combination of intravenous and inhaled therapy was superior, with an improved FEV<sub>1</sub>, FVC, PEF and a more than 6 fold reduction in sputum volume (el-Din et al., 1994). More recently, a double blind, multicentre trial comparing 14 days treatment with either inhaled tobramycin and oral ciprofloxacin with oral ciprofloxacin alone in 53 patients known to be chronically colonised with *Pseudomonas aeruginosa* suffering an acute exacerbation found that with inhaled tobramycin, there was a greater reduction in sputum bacterial density,

although improvements in other clinical outcomes measured were not found to be superior (Bilton et al., 2006).

The studies to date have generally used empirical, broad spectrum antibiotic therapy proven for *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Staphylococcus aureus*. To date, there are no randomised controlled trials comparing antibiotics with placebo in the treatment of acute exacerbations of bronchiectasis, but consensus opinion for selection is suggested in Table 3 below.

Table 3. Recommended antibiotic therapy for exacerbations of bronchiectasis according to pathogenic organism (adapted from BTS Guideline (Pasteur et al., 2010).

<b>Organism</b>	<b>First Line Treatment</b>	<b>Length of Treatment</b>	<b>Second Line Treatment</b>	<b>Length of Treatment</b>
<i>Streptococcus pneumoniae</i>	Amoxicillin 500mg TDS	14 days	Clarithromycin 500mg BD	14 days
<i>Haemophilus influenzae</i> ( $\beta$ lactamase negative)	Amoxicillin 500mg- 1G TDS (3G BD in severe disease)	14 days	Clarithromycin 500mg BD Ciprofloxacin 500mg BD Ceftriaxone 1-2g OD (IV)	14 days
<i>Haemophilus influenzae</i> ( $\beta$ lactamase positive)	Co-amoxiclav 625mg TDS	14 days	Clarithromycin 500mg BD Ciprofloxacin 500mg BD Ceftriaxone 1-2g OD (IV)	14 days
<i>Moraxella catarrhalis</i>	Co-amoxiclav 625mg TDS	14 days	Ciprofloxacin 500mg BD	14 days
<i>Staphylococcus aureus</i>	Flucloxacillin 500mg QDS	14 days	Clarithromycin 500mg BD	14 days
<b>Methicillin resistant</b> <i>Staphylococcus aureus</i> <b>Oral Therapy</b>	<50Kg: Rifampicin 450mg + trimethoprim 200mg BD >50Kg: Rifampicin 600mg + trimethoprim 200mg BD	14 days	<50Kg: Rifampicin 450mg + doxycycline 200mg OD >50Kg: Rifampicin 600mg + doxycycline 200mg OD	14 days

Organism	First Line Treatment	Length of Treatment	Second Line Treatment	Length of Treatment
<b>Methicillin resistant</b> <i>Staphylococcus aureus</i> <b>IV Therapy</b> <b>(Doses see BNF)</b>	Vancomycin Or Teicoplanin	14 days	Linezolid	14 days
<b>Coliforms</b> <b>(eg <i>Klebsiella</i>, <i>Enterobacter</i>)</b>	Ciprofloxacin 500mg BD	14 days	Ceftriaxone 1-2g (IV)	14 days
<i>Pseudomonas aeruginosa</i>	Ciprofloxacin 500mg BD  (Or, in severe infections Ciprofloxacin 750mg BD)	14 days	Monotherapy (IV): Ceftazidime 2g TDS Or Tazocin 4.5g TDS Or Aztreonam 2g TDS Or Meropenem 2g TDS  Dual therapy: Each of the above may be combined with either: gentamicin or tobramycin or colomycin	14 days

<b>Organism</b>	<b>First Line Treatment</b>	<b>Length of Treatment</b>	<b>Second Line Treatment</b>	<b>Length of Treatment</b>
<b>Mixed Normal Flora Or not chronically infected</b>	Amoxicillin 500mg TDS	14 days	Clarithromycin 500mg BD	14 days



### *Adjunctive therapies in acute exacerbations*

There are few studies that have explored the role of adjunctive therapies in acute exacerbations of bronchiectasis.

There have been no randomised placebo controlled trials evaluating the efficacy of chest physiotherapy in acute exacerbations but studies have compared different chest physiotherapy techniques during exacerbations. A single blind trial randomised 15 patients with an acute exacerbation to either postural drainage and breath/cough manoeuvres, the flutter device and breath/cough manoeuvres or to breath/cough manoeuvres alone and found no difference in sputum weight, FEV<sub>1</sub>, FVC or PEFR between any of the groups (Tsang, 2003). A later study randomised 20 patients to either the Acapella device or to continue their usual physiotherapy technique again found no significant difference between the groups in terms of sputum volume produced, patient perception of dyspnoea or spirometry (Patterson et al., 2007).

There has been one study exploring bromhexidene as a mucolytic adjunctive in the management of inpatient exacerbations. A randomised double blind placebo controlled trial of bromhexidene 300mg three times daily in addition to intravenous ceftazidime improved ease of expectoration and increased sputum production (Olivieri et al., 1991).

The outcomes of patients with exacerbations of bronchiectasis and acute respiratory failure requiring management with either non-invasive ventilation or invasive mechanical ventilation have been reviewed in two retrospective studies (Dupont et al., 2004, Phua et al., 2010). In the first study, 48 patients with acute exacerbations of bronchiectasis and acute respiratory failure were admitted to intensive care over the ten

year study review period between 1990 and 2000, 13 of whom required non-invasive ventilation and 26 of whom required invasive mechanical ventilation. The intensive care unit mortality was 19% and one year mortality was 40%, with factors associated with reduced survival including prior use of long term domiciliary oxygen therapy and age greater than 65 years (Dupont et al., 2004). The more recent study found similar outcomes in 57 patients admitted to the intensive care unit over an 8 year period. 31 patients were managed with non-invasive ventilation and 26 with invasive mechanical ventilation. There was no difference in mortality between the groups (25.8% in the non-invasive ventilation group and 26.9% in the invasive mechanical ventilation group) and this was predicted by the APACHE II score. There have been no randomised controlled trials to evaluate the role of non-invasive ventilation with invasive mechanical ventilation in acute exacerbations of bronchiectasis.

The role of other therapeutic strategies have not been studied in acute exacerbations, particularly the use of corticosteroids (either systemic or inhaled), other airway clearance techniques such as hypertonic nebulised saline, airways humidification and oxygen and further studies are needed.

## **1.9     Assessing response to treatment**

Treatment efficacy is typically measured by the ability to halt the pathogenesis of the disease. In chronic conditions, defining treatment efficacy necessitates encompassing the intent of treatment: to slow disease progression and to improve patients' health related quality of life. In bronchiectasis, treatment goals frequently include improving symptoms, reducing exacerbation frequency and severity as well as limiting the bacterial burden and inflammatory insult in the airways.

Currently, few clinical or laboratory markers specifically validated for bronchiectasis exist and it is poorly understood how best to assess the disease and its response to treatment. Early studies relied predominantly on subjective non-quantitative measures such as "the foetor of the breath and sputum" to assess treatment success (Grainger-Stewart, 1893). More recent interventional studies have used a variety of clinical and laboratory parameters to monitor response to treatment, many of which have been selected based on their utility in other chronic respiratory disease processes. Laboratory markers used in studies have included qualitative and quantitative sputum bacteriology as well as sputum and serum inflammatory measures. Clinical indices used include 24hour sputum volume, sputum purulence, lung function (typically FEV<sub>1</sub>, FVC and PEF<sub>R</sub>), symptoms scores and in longer-term studies, frequency and severity of exacerbations. To date, there are only two validated measures in non-cystic fibrosis bronchiectasis that have been proven to be responsive to change- the St George's Respiratory Questionnaire and the Leicester Cough Questionnaire (LCQ) (Wilson et al., 1997a, Murray et al., 2009d).

### *Laboratory markers*

Bronchiectasis is characterised by neutrophilic airways inflammation and it has been hypothesised that direct measures of lung inflammation using markers in exhaled breath and sputum may be useful in assessing disease activity and response to treatment. Interventional studies of antibiotic therapy have explored the effect of treatment on various markers of neutrophilic inflammation including sputum neutrophil elastase, myeloperoxidase and pro-inflammatory cytokines such as IL1 $\alpha$ , IL8, TNF $\alpha$  and LTB4 (Stockley et al., 1984a, Hill et al., 1988). Sputum elastase levels have been shown to reduce with one week of antibiotic treatment in acute exacerbations and also in stable disease treated with longer term antibiotics (Stockley et al., 1984a, Hill et al., 1988). Myeloperoxidase levels were also found to reduce with a short course of nebulised antibiotic treatment in stable disease (Lin et al., 1997). A longer study exploring the impact of 8 weeks of erythromycin treatment in stable disease found no effect on the proinflammatory cytokines IL1 $\alpha$ , IL8, TNF $\alpha$  and LTB4 (Tsang et al., 1999b). The full potential of such markers requires exploration however, as none have been validated as measures of response to treatment. Their clinical utility as cost effective, reliable and pertinent markers also requires full assessment.

Studies have investigated other markers of airways inflammation that may have a role in assessing disease activity in bronchiectasis, particularly those with proven utility in other chronic inflammatory respiratory diseases including exhaled hydrogen peroxide, exhaled nitric oxide and serum procalcitonin, although there have been no formal validation studies of any such markers. Exhaled breath hydrogen peroxide was analysed in a cross-sectional study of 30 patients with bronchiectasis and found to be present in

higher concentrations than in healthy controls [1.1(0.87-1.29) $\mu$ Mol versus 0.3(0.19-0.36)  $\mu$ Mol] (Loukides et al., 2002). Within the bronchiectasis patients, higher levels were found in those chronically infected with *Pseudomonas aeruginosa* and in those requiring management with long term antibiotics. Significant positive correlations were seen with the concentration of hydrogen peroxide and symptoms score, percentage of neutrophils in the sputum and radiological extent of disease as well as a negative correlation with FEV<sub>1</sub>. Despite these findings suggesting that exhaled breath hydrogen peroxide could be a useful measure in bronchiectasis, its utility has not been further explored in interventional studies.

Peripheral airway nitric oxide has been found to be a useful direct measure of inflammation in other chronic respiratory diseases such as asthma (Barnes et al., 2010). A recent study has explored its potential utility in bronchiectasis, finding higher levels in patients with bronchiectasis than in controls [3.6(2.1)ppb versus 2.7(1.5)ppb respectively] (Shoemark et al., 2011). The levels of fractional exhaled nitric oxide (FeNO) were found to correlate with the severity of bronchiectasis using lung function, radiological parameters and quality of life scores. Its role as a marker of response to treatment is less clear, as no difference was found in levels of FeNO following treatment for an acute exacerbation in 20 patients (Shoemark et al., 2011). Procalcitonin, the peptide precursor to the hormone calcitonin is a useful marker of severity in pneumonia and other sepsis syndromes. However, a study of its utility in exacerbations of bronchiectasis found that it is unlikely to be useful with generally low serum levels and in patients requiring inpatient management, there was no correlation between serum procalcitonin and other inflammatory markers (Loebinger et al., 2008).

Only a limited number of possible biomarkers for monitoring disease and response to treatment in bronchiectasis have been studied. A recent study involving 19 patients with bronchiectasis used the mass spectrometry technique surface enhanced laser desorption ionisation time of flight (SELDI-TOF) to actively select proteins from sputum using adherence to chromatographic surfaces with subsequent mass spectrometry and found 377 potential biomarkers may exist within sputum from patients with bronchiectasis compared with healthy controls (Gray et al., 2008). A later study analysed 16 samples of sputum for trace metals and found significantly higher levels of zinc, iron and manganese compared with controls (Gray et al., 2010). More work is needed to identify markers specific to bronchiectasis, relevant to pathogenesis and responsive to changes in disease activity.

The airways in bronchiectasis are frequently chronically infected with pathogenic bacteria and interventional studies have therefore used sputum bacterial clearance as an important marker of treatment efficacy (Davies et al., 1983, Lam et al., 1989, Pines et al., 1974, Drobic et al., 2005, Currie et al., 1990). The majority of antibiotic studies have relied upon qualitative sputum bacteriology (described as bacterial eradication or clearance) as an outcome measure but in a disease characterised by chronic airways colonisation, treatment response may be more sensitively measured by quantitative bacteriology which has only been used to a limited extent in interventional studies to date (Tsang et al., 1999b). Bacterial density in the airways in bronchiectasis is crucial to disease pathogenesis with increasing load associated with a significant increase in airways inflammation (Angrill et al., 2001, Hill et al., 2000). Further studies are needed to test this hypothesis and validate quantitative bacteriology as an important measure of

treatment response, superior to solely using qualitative bacteriology and bacterial clearance particularly to warrant the associated greater clinical cost.

### *Clinical Markers*

Interventional studies in bronchiectasis have typically used a variety of clinical outcome measures to assess treatment response. Despite this, there remains no single uniformly agreed optimum measure in terms of reliability, relevance and cost. One of the most frequently used clinical outcome measures is sputum colour with the majority of interventional antibiotic studies using conversion of purulent to mucopurulent or mucoid sputum as a reflection of treatment efficacy (Stockley et al., 1984a, Lam et al., 1986, 1957, Pines et al., 1967). A reduction in sputum volume has also been used as a marker of treatment success in antibiotic studies although conversely physiotherapy studies have suggested that increased sputum volume is a useful marker of treatment response. Lung function indices (most commonly FEV<sub>1</sub>, FVC, PEFR and TLCO) have also been used as outcomes in antibiotic, inhaled corticosteroid and physiotherapy studies but their response to these interventions has been variable with studies frequently observing minimal change or indeed statistical changes that may have little clinical relevance (Thompson et al., 2002, Tsang et al., 2005a). Respiratory muscle pressures in bronchiectasis have recently been studied in 20 patients with clinically stable disease, with maximal inspiratory pressure demonstrated to have reliability, suggesting that there may be a role for it as a clinical outcome measure, but further studies are needed (Moran et al., 2010). Exercise capacity has also been used as a potentially useful marker in interventional studies (Lin et al., 1997) and the 6minute walking test has been demonstrated to be strongly associated with health related quality

of life in bronchiectasis and radiological extent of disease (defined by the generations of bronchial divisions affected) but again has not been validated for use as a parameter of treatment response (Lee et al., 2009).

In longer term studies the number, frequency and severity of acute exacerbations has been used to measure treatment success. This encompasses one of the main aims of disease management in bronchiectasis, reducing further insults to the airways that may contribute to disease progression. Studies have also used symptoms as a guide to treatment efficacy. The earliest interventional studies relied on patient reporting which was difficult to quantify and use for comparison between studies (Davies et al., 1983, Lam et al., 1986). The only currently validated measures of treatment response in bronchiectasis are both measures of health related quality of life- the St George's Respiratory Questionnaire and the Leicester Cough Questionnaire.

The St George's Respiratory Questionnaire is a 50 item self-administered health related quality of life questionnaire assessing the impact of multiple chronic respiratory symptoms on HRQL, originally intended for use in COPD (Jones et al., 1992). It consists of 3 components- symptoms (8 items), activity (16 items) and impacts (26 items) and assesses the impact of symptoms over the preceding 4 weeks. In bronchiectasis it has been shown to have repeatability over a 2 week period with an intraclass correlation coefficient of 0.97. Responsiveness of the SGRQ was validated by assessing change in score compared with parameters such as the level achieved in the shuttle walk test, MRC dyspnoea score and Short Form 36 HRQL score over a 6 month period (Wilson et al., 1997a).



The Leicester Cough Questionnaire is a 19 item self-completed quality of life measure of chronic cough (Birring et al., 2003). It has 3 domains: physical (8 items), psychological (7 items) and social (4 items). The total severity score ranges from 3-21, with a lower score indicating greater impairment of health status due to cough. It assesses the impact of symptoms over the preceding two weeks. The validation of the questionnaire was undertaken prior to the commencement of the studies reported in this thesis and is described in the General Methods section (Murray et al., 2009d).

For a parameter to be useful in disease management, it must be pertinent to the condition, non-invasive, inexpensive, easily accessible, reliable and responsive to change. There are few such markers currently available for non-cystic fibrosis bronchiectasis and much work is needed to allow any progress to be made in the development and implementation of potential therapies.

### **1.10 Aims**

There were two main aims to this thesis:

1. To explore whether the vicious cycle of inflammation and infection in bronchiectasis can be disrupted and whether this impacts clinically on the symptoms of bronchiectasis.
2. To identify potentially useful clinical markers for monitoring treatment response in patients with non-cystic fibrosis bronchiectasis.

The first aim involved designing and conducting three separate studies.

1. Currently, regular chest physiotherapy is advised as a mainstay of management in bronchiectasis. The rationale for its use is to help mobilise and clear secretions with their debris and bacteria from the damaged airways. Although various physiotherapy techniques are practised, evidence for its efficacy is limited. The first study was designed to assess the efficacy of regular chest physiotherapy as treatment strategy in its own right- does augmentation of the impaired mucociliary transport escalator disrupt the vicious cycle of infection and inflammation and can it improve the symptoms of bronchiectasis?
2. The second study was designed to assess the impact of reducing bacterial infection in the airways on both clinical and laboratory outcomes during acute exacerbations of bronchiectasis- can infection and inflammation be reduced and clinical improvement achieved?
3. The third study was designed to assess whether limiting the bacterial burden in the airways on a long-term basis can reduce airways inflammation, disrupting the vicious cycle of inflammation and infection in the airways. The second

question this study sought to address was if the vicious cycle of infection and inflammation can be disrupted does this lead to clinical and symptomatic benefits? A randomised controlled trial of nebulised antibiotics administered over a 12 month period was therefore conducted.

The second aim sought to explore whether there are any key parameter- either clinical or laboratory markers- that can be used to assess treatment response in the management of bronchiectasis. The endpoints used in the first and third studies conducted as part of the first aim were analysed.

**CHAPTER TWO:**  
**GENERAL METHODS**

## **2.1 Ethical approval and consent**

All studies included in this thesis had approval from the local research ethics committee for NHS Lothian or where applicable, the Scottish Multi-Centre Research Ethics Committee. All patients provided written consent.

## **2.2 Patients**

All patients were recruited from the specialist bronchiectasis clinic, Royal Infirmary of Edinburgh, NHS Lothian. All had both a clinical and radiological diagnosis of bronchiectasis.

### *Clinical diagnosis*

Patients had a history of a chronic cough with daily sputum expectoration and suffered at least two exacerbations requiring antibiotics in the preceding year. All had chronically infected sputum which was defined as the culture of pathogenic organisms in at least three sputum samples when clinically stable in the preceding twelve months.

### *Radiological diagnosis*

All patients had a high resolution computed tomography (HRCT) scan of the thorax with bronchiectasis diagnosed if there was evidence of bronchial dilatation, defined as the diameter of the bronchial lumen being greater than the luminal diameter of the adjacent pulmonary artery, with a lack of tapering on sequential slices (Naidich et al., 1982).

For the studies reported in this thesis, any patient with a clinical and radiological diagnosis of bronchiectasis but who also had any of the following, were excluded:

current smokers; ex-smokers of less than two years; cystic fibrosis (cystic fibrosis transmembrane receptor sequences present on genotyping); active pulmonary mycobacterial infection; active sarcoidosis; active allergic bronchopulmonary aspergillosis (ABPA); chronic obstructive pulmonary disease (COPD) (Celli and MacNee, 2004); poorly controlled asthma (poorly controlled asthma was defined objectively as a 20% or more diurnal variation in peak expiratory flows despite treatment); long term regular oral corticosteroid treatment; long term regular antibiotic treatment (oral or inhaled).

For the first two studies reported in this thesis, patients were required to be clinically stable at the point of entry to the study. In the four weeks preceding entry to the study, there had to be no changes in routine respiratory medication and no requirement for antibiotics or corticosteroid treatment.

### **2.3 Sputum collection**

Two different types of sputum collection were used throughout the studies, early morning samples and 24 hour collections.

Early morning samples: all sputum expectorated in the first four hours on rising from bed on the day of assessment was collected in a sterile, transparent container.

24 hour sputum collections: all sputum expectorated in the 24hours preceding the study visit was collected in a sterile, transparent, pre-calibrated container.

Sputum samples were all visually analysed immediately upon receipt as described below.

## **2.4 Sputum characteristics**

Each sputum sample was assessed for both colour and volume.

### *Sputum colour*

A sputum colour chart specific to bronchiectasis was used to describe the three typical gradations of sputum colour: mucoid (clear), mucopurulent (pale yellow/pale green) and purulent (dark yellow/dark green) (Murray et al., 2009b) (Appendix 9.1). The chart was developed and piloted in the bronchiectasis clinic at the Royal Infirmary of Edinburgh prior to the commencement of the studies reported in this thesis to ensure consistency of reporting between staff and patients as well as to ensure patients were confident in using it to describe their symptoms. 141 individual patients' sputum samples were graded using the colour chart by both doctor and patient. Agreement was 80.9% in the mucoid group, 90.3% in the mucopurulent group and 76.3% in the purulent group. In all cases of disagreement except in 5 (21.7%), the doctor interpretation was of greater purulence than the patient interpretation. The intraclass correlation coefficient was 0.83, 95% confidence interval 0.76-0.87,  $P < 0.0001$  and Cronbach's alpha coefficient for comparing the Doctor interpretation with Patient interpretation was 0.91.

### *Sputum volume*

Sputum was collected in sterile, transparent, calibrated containers. The containers were calibrated using water. The total volume was read directly from the meniscus. The volume was recorded for both early morning samples and 24 hour collections. This method has not been externally validated but is accepted as standard in clinical practice.

## **2.5    Sputum analysis**

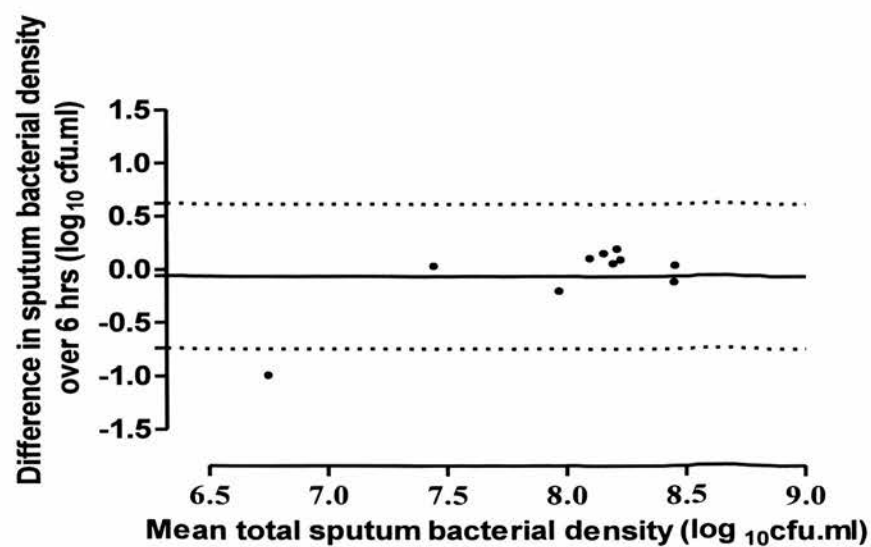
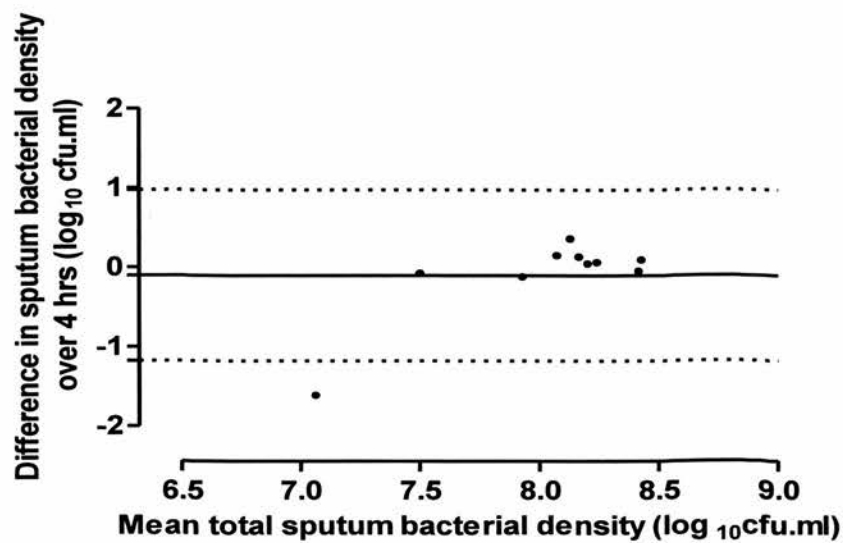
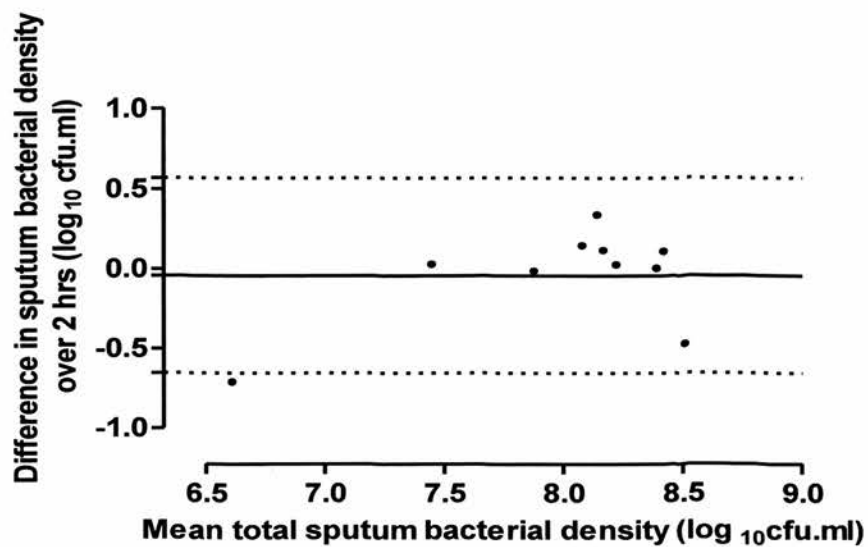
All sputum samples were processed as soon as possible following collection.

Prior to the commencement of the studies reported in this thesis, a study was performed to investigate the effects of storage and processing of sputum samples (Murray et al., 2010). This pilot study was conducted in 10 patients colonised with *Pseudomonas aeruginosa*. Figure 6 demonstrates that there was no significant difference ( $P=0.4$ ) in *Ps. aeruginosa* density when processing the sputum at 1hr, 2hrs, 4hrs and 6hrs from expectoration. The mean difference (standard deviation) with 95% limits of agreement between processing at 1hr compared with 2hrs was  $-0.04(0.31)$ ,  $-0.65$  to  $0.57 \log_{10}$  colony forming units/millilitre ( $\log_{10}$  cfu/ml); between 1 hr and 4hrs was  $-0.1(0.54)$ ,  $-1.2$  to  $0.97$  and between 1hr and 6hrs was  $-0.06(0.35)$ ,  $-0.7$  to  $0.6$ .

Figure 6. Change in sputum bacterial density with processing of sputum up to six hours post expectoration.

The figure shows a Bland-Altman plot of sputum bacterial density over 2hrs, 4hrs and 6hrs. The solid line represents the mean difference between the density at 1hour post expectoration and 2hrs, 4hrs and 6hrs respectively. The perforated lines represent the upper and lower 95% limits of agreement.





## **2.6     Sputum Gram staining**

A 10µl sterile loop was used to transfer sputum to a clean, dry, frosted glass slide. 10µl of sterile saline was gently pipetted onto the sputum and the sample smeared uniformly and thinly across the slide. The sample was air dried and subsequently heat fixed by passing the slide through a low flame several times until all moisture had evaporated. The slide was then flooded with the primary staining reagent methyl violet for one minute followed by gentle rinsing in deionized water. The mordant iodine was then used to flood the slide for one minute followed by further gentle rinsing in deionized water. Following draining, acetone was used as a decolourising agent, flooding the slide until the fluid appeared colourless (approximately ten seconds). The slide was immediately rinsed in deionized water. The counterstain basic fuchsin was then used to flood the slide for one minute before further rinsing in deionized water. The slide was gently blotted using paper towel and allowed to air dry. The results of the staining procedure were observed under oil immersion microscopy. At low (x100) magnification, the number of squamous cells and polymorphonuclear leukocytes per field were counted. At high (x1000) magnification, bacterial cell colour was described as purple (Gram positive) or pink (Gram negative) and morphology was noted (rods or cocci).

All sputum samples were considered to be valid sputum if there were >25 polymorphonuclear leukocytes and <10 squamous cells on Gram stain per low-power (x100 magnification) field (Gleckman et al., 1988).

## **2.7 Quantitative sputum bacteriology**

Sputum was prepared for quantitative bacteriology. A minimum of one millilitre of fresh sputum was used. An equal volume (1:1) of 0.1% dithiothreitol was added to the sputum sample and vortexed vigorously. The liquefied, homogenised sample was serially diluted using 0.9% sterile saline to achieve dilutional factors of  $10^{-1}$  to  $10^{-4}$ . 100ul aliquots of diluted homogenised sputum (neat, 1/100 and 1/10000) were then inoculated across the entire surface of the following agar selective or enrichment media using a sterile “hockey stick” spreader (Pye et al., 1995): Cefrimide (Difco) *Pseudomonas* isolation agar, chocolate with bacitracin agar and horse blood agar. The inoculated cefrimide and horse blood agars were incubated aerobically at 37°C for 48hours and the inoculated chocolate with bacitracin agars were incubated at 5% CO<sub>2</sub> at 37°C for 48hours. Colony forming units for each pathogen were identified and counted at 48hours for each pathogen. The sputum bacterial density was expressed as log<sub>10</sub> colony forming units per millilitre (log<sub>10</sub> cfu/ml).

## **2.8 Qualitative sputum bacteriology**

Viable pathogens were identified following incubation based on colonial morphology, Gram's stain and further, specific standardised identification tests. The identification methods used for the most common organisms cultured is described.

### *Haemophilus influenzae*

The primary isolation media used for *Haemophilus influenzae* was chocolate with bacitracin agar incubated at 37°C at 5%CO<sub>2</sub>. Colonies are small, round and convex and typically appear after 24hrs incubation. The species appear negative on Gram staining as spherical, oval or rod shaped cells of less than 1µm diameter. Colonies suspected to be *Haemophilus* species from morphology and Gram's stain appearance were further identified based on their requirement for X and V factors. One or more colonies were selected using a sterile straight wire loop and emulsified in distilled water to produce a light suspension. 100µl of suspension was inoculated onto nutrient agar and spread evenly using a sterile hockey stick spreader. Three filter paper discs incorporating X factor (comprising protoporphyrin IX and haemin), Y factor (comprising nicotinamide adenine dinucleotide) and X and Y factors together respectively, were positioned on the inoculated agar surface in the configuration of an equilateral triangle with a minimum of 3.5cm distance between the discs. Following overnight incubation at 37°C, the agar plate was examined for growth around the discs. *Haemophilus influenzae* was distinguished from *Haemophilus parainfluenzae* as detailed in Table 4.

Table 4. Identification of *Haemophilus influenzae* and *Haemophilus parainfluenzae*.

<b>Factor</b>	<b><i>Haemophilus influenzae</i></b>	<b><i>Haemophilus parainfluenzae</i></b>
<b>X</b>	Negative for growth around disc	Negative for growth around disc
<b>V</b>	Negative for growth around disc	Positive for growth around disc
<b>X and V</b>	Positive for growth around disc	Negative for growth around disc

X and V test results for *Haemophilus influenzae* and *Haemophilus parainfluenzae*.

### *Moraxella catarrhalis*

The primary isolation media used for *Moraxella* species was horse blood agar. Colonies are white or buff and convex in shape. The species appear as negative cocci on Gram's stain, are approximately 0.6-1.0µm diameter, and occur either singly or in pairs. Colonies suspected to be *Moraxella* species from morphology and Gram's stain appearance were tested for a positive oxidase reaction. Filter paper was soaked in the test reagent N, N, N', N'-tetra-methyl-p-phenylenediamine dihydrochloride. A sterile wooden stick was used to select at least one suspicious colony and rub it onto the pre-soaked filter paper. A reaction was looked for within ten seconds. A positive result was confirmed with the development of a blue colour, indicating oxidase production. A negative result was indicated by an absence of colour.

### *Streptococcus pneumoniae*

The primary isolation media used for *Streptococcus* species was horse blood agar. Colonies appear white, 1-2mm in diameter and may have a classic draughtsman appearance due to autolysis after incubation. *Streptococcus* species are positive on Gram's staining, appear round and are in pairs, chains or clusters. Colonies suspected from morphology and Gram's stain appearance to be *Streptococcus pneumoniae* were further identified by assessing their sensitivity to ethylhydrocupreine hydrochloride (optochin test). Ethylhydrocupreine hydrochloride causes changes in surface tension of the cell membrane causing *Streptococcus pneumoniae* to lyse. Fresh suspicious colonies were selected using a sterile straight wire loop and streaked across a horse blood agar plate. An optochin disc (filter paper disc impregnated with 5µg of ethylhydrocupreine hydrochloride) was placed in the centre of the inoculated agar and incubated at 37°C at

5%CO<sub>2</sub> for 24hours. Following incubation, the inoculated agar was examined for zones of inhibition. A positive result was defined as a radial zone of inhibition measuring 5mm or more from the edge of the disc. A negative result was defined as either no zone of inhibition or a zone of inhibition less than 5mm radius from the edge of the disc.

### *Staphylococcus aureus*

The primary isolation media used for *Staphylococcus aureus* was horse blood agar. Colonies are opaque with either a creamy white colour or a yellow-orange colour. Gram staining shows Gram positive cocci occurring either singly, in pairs or in irregular clusters. Colonies suspected to be *Staphylococcus aureus* were confirmed using the commercial test kit Dryspot Staphytect Plus, a latex slide agglutination test according to the manufacturer's instructions (Oxoid Limited, Basingstoke, Hampshire).

### *Pseudomonas aeruginosa*

Cetrimide (Difco) *Pseudomonas* isolation agar was used as the primary selective media for *Pseudomonas aeruginosa*. Morphological identification of colonies may have included several characteristics. The most common type of colony is a large, low oval convex shape with a rough appearance. There may be a characteristic smell of aminoacetophenone and colonies may have a blue-green appearance due to the production of pyocyanin (blue) and pyoverdin (yellow). The production of this pigment is indicative of *Pseudomonas aeruginosa*, although some strains particularly mucoid strains may not produce pyocyanin. Further identification of colonies was confirmed using the commercially available API20NE kit (bioMerieux UK Limited, Basingstoke, Hampshire) according to the manufacturers' instructions. Briefly, approximately one to

four colonies were selected using a sterile straight wire loop and emulsified with 2ml of 0.85% sterile saline to produce a suspension approximating a 0.5McFarland standard. The API20NE test strip consists of twenty microtubes containing dehydrated substances. These microtubes were inoculated with the bacterial suspension and incubated for 24 or 48 hours at 30°C with the addition of reagents and interpretation of reactions done according to the manufacturer's directions. The biochemical reactions were converted accordingly into an octal profile number and decoded using the Analytical Profile Index (API Database Vn6.0, APILAB Software Vn3.3.3, Apilab Plus; bioMerieux).

#### *Enterobacteriaceae*

*Enterobacteriaceae* were selected from horse blood agar. The colonies are 2-3mm in diameter, are low, convex, grey and maybe smooth or mucoid. The cells appear as Gram negative rods. Further identification of suspicious colonies was initially with a negative oxidase test as described previously and then the commercial kit API20E was used (bioMerieux UK Limited, Basingstoke, Hampshire). The principle and preparation of the API20E strip is similar to the API20NE strip described above, with incubation for only 24hours at 30°C.



## **2.9**    **Sputum biochemical assays**

Sputum was ultra centrifuged at 50,000×g for 90 minutes at 4°C. The sol phase was removed and immediately frozen at -70°C until required for analysis.

### *Sputum myeloperoxidase*

Sputum myeloperoxidase (MPO) activity was used as a measure of neutrophil influx. MPO activity was measured from sputum sol phase by chromogenic substrate assay. Standards and samples were diluted as necessary in phosphate buffered solution. Twenty-five microlitres of standard or sample were added to the wells of a microtitre plate (Serotec), followed by twenty-five microlitres of tetramethylbenzidine (TMB). The plate was then incubated for 5minutes at 25°C. Fifty microlitres of sulphuric acid solution was added to each well at the end of the incubation period and absorbance was measured using a dual wavelength of 450 and 560nm and the MPO concentration interpolated from the standard curve and expressed as µg/ml. The MPO concentration was determined in duplicate for each sample or standard and the mean determined for each.

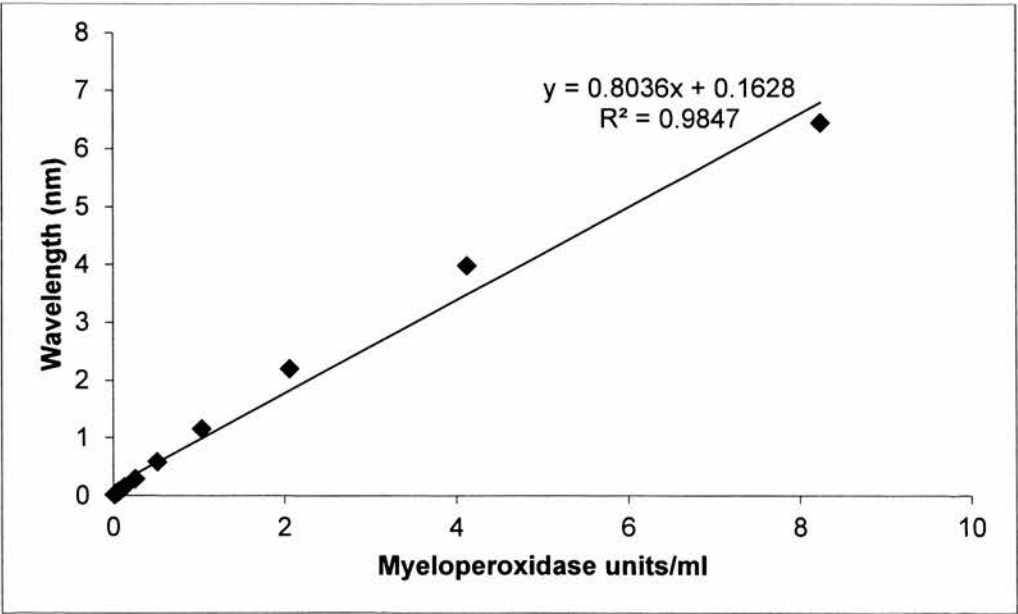
The standards, sample dilution used, and the lower detection of the assay is illustrated in Table 5. The standard curve is shown in Figure 7.

Table 5. Sputum neutrophil myeloperoxidase assay.

Standards	Typical Sample Dilution	Lower Limit of Detection
0.008-8.25units/ml	1 in 20 to 1 in 500	0.016units/ml

The standards, sample dilutions and lower limits of detection used for the myeloperoxidase assay.

Figure 7. Sputum neutrophil myeloperoxidase assay standard curve.



### *Sputum neutrophil elastase*

Sputum neutrophil elastase was used as a measure of neutrophil influx. Neutrophil elastase activity present in the samples was measured spectrophotometrically as the rate of change of optical density at 405nm at 37°C using the chromogenic peptide substrate methoxysuccinyl-ala-ala-pro-val-paranitroanilide (MeoSAAPvN, Elastin Products, UK). This rate of change was compared with a standard curve for the rate of change in optical density, obtained by incubating known concentrations of human neutrophil elastase (Sigma Aldrich, UK) with the same chromogen. The human neutrophil elastase lyophilized powder was reconstituted in sodium acetate 50mM, pH5.56 + 200mM NaCl. Standards and samples were diluted as necessary in 50mM [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (HEPES) + 150mM NaCl + 0.05% Igepal CA630, pH8. Forty microlitres of standard or sample were added to each well of a 96 well microtitre plate (Nunc). 40µl of MeoSAAPvN was then added to each well and the samples immediately read at 37°C for a minimum of thirty minutes with readings every two minutes. The rate of change in optical density was converted into elastase activity and expressed in units per milligram. The elastase concentration for each sample was determined in duplicate and the mean determined for each.

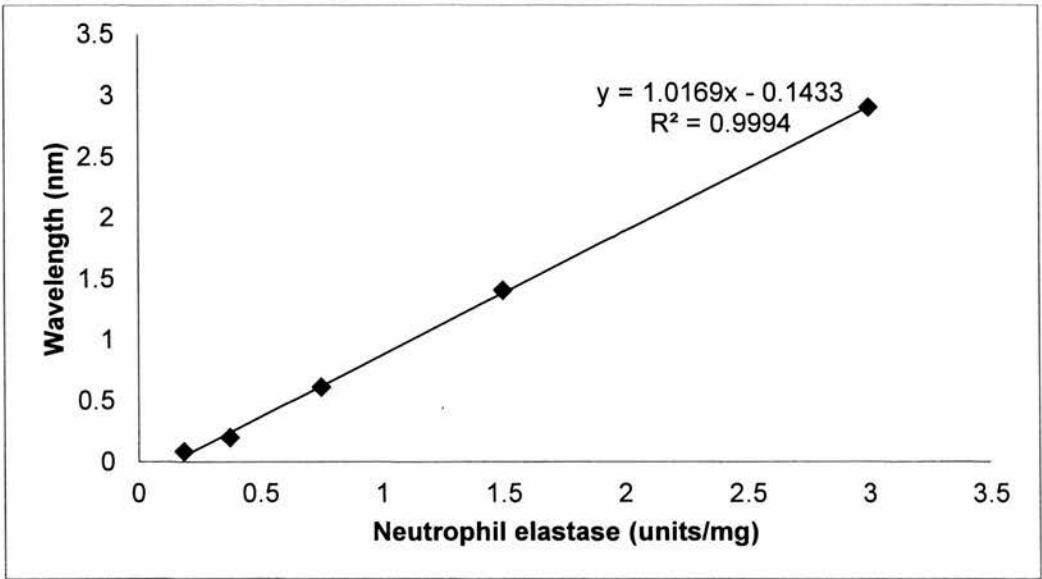
The standards, sample dilution used, and the lower detection of the assay are illustrated in Table 6. The standard curve is shown in Figure 8.

Table 6. Sputum neutrophil elastase assay.

Standards	Typical Sample Dilution	Lower Limit of Detection
0.002-3.0 units/mg	1 in 20 to 1 in 500	0.18units/mg

The standards, sample dilutions and lower limits of detection used for the neutrophil elastase assay.

Figure 8. Sputum neutrophil elastase assay standard curve.



## **2.10 Serum samples**

### *Systemic inflammation*

15mls of venous blood was collected and analysed for total leucocyte count (WCC) expressed as  $\times 10^9$  cells/l; normal range 4-11 $\times 10^9$ /l; erythrocyte sedimentation rate (ESR) measured in mm/hr with normal range for males calculated as age/2 and for females age + 10/2, and C-reactive protein (CRP) measured in mg/l, with the accepted normal range <7mg/l.

### *Routine monitoring*

The interventional nature of the studies conducted for this thesis required monitoring for potential adverse systemic effects. All patients therefore routinely had venous blood taken for measurement of blood biochemistry including: urea [mmol/l; normal range 2.5-6.6]; creatinine ( $\mu$ mol/l; normal range 60-120], sodium (mmol/l; normal range 135-145) and potassium (mmol/l; normal range 3.6-5.0) and monitoring of haematological indices: haemoglobin (g/l; normal range for males 130-180 and for females 115-160) and platelet count ( $\times 10^9$ /l; normal range 150-350). Other monitoring tests conducted routinely specific to the interventions are reported in the methods section of the individual chapters.

### **2.11 HRCT thorax scoring**

Assessment of bronchiectasis was by evaluation of the bronchi on a lobar basis (for the purposes of the studies included in this thesis the lingula was counted as a separate lobe). The number of lobes affected was recorded and severity of bronchial dilatation was graded 1-3 according to the Reiff criteria: grade one: less than twice the diameter of adjacent pulmonary artery (described as cylindrical); grade two: two to three times the diameter of adjacent pulmonary artery (described as varicose); grade three: greater than three diameter of the adjacent pulmonary artery (described as cystic), giving a maximum possible total score for six lobes of eighteen (Reiff et al., 1995).

## **2.12 Pulmonary Function Tests**

Forced Expiratory Volume in One Second ( $FEV_1$ ), Forced Vital Capacity (FVC) and mid-expiratory flows ( $FEF_{25-75}$ ) were measured according to standardised guidelines (ERJ 2005) using a MicroMedical Microloop ML3535 (Viasys Healthcare).

$FEV_1$  is the maximal volume of air exhaled in the first second of forced expiration following a forced expiration from a position of full inspiration and is expressed in litres. FVC is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration and is expressed in litres. The highest of three technically satisfactory measurements (within 10%) was recorded for each and the  $FEV_1/FVC$  ratio was calculated.  $FEF_{25-75\%}$  is the mean forced expiratory flow between 25% and 75% of the FVC and was calculated from the blow with the largest sum of FVC and  $FEV_1$ . It is expressed as litres/second. Actual values were recorded and were also expressed as % predicted for the patients age, sex, and height.

Respiratory muscle strength was assessed by measuring the Maximum Inspiratory Pressure (MIP) and Maximum Expiratory Pressure (MEP) using the MicroRPM (Respiratory Pressure Meter) (Viasys Healthcare) with the highest of three technically satisfactory measurements recorded for each. The MIP was measured following complete exhalation to near residual volume followed by maximal deep inspiration against the occluded airway (Mueller manoeuvre) and expressed as  $cmH_2O$ . The MEP was measured following deep inspiration to near total lung capacity followed by strong expiration (Valsalva manoeuvre) and expressed as  $cmH_2O$ . Actual values were recorded and were also expressed as % predicted for the patients age, sex, and height.

### **2.13 Exercise capacity**

The incremental shuttle walk test (ISWT) was used to assess functional exercise capacity. It has been proven in patients with COPD to provide an objective measure of disability and can be used for direct comparison of patients' performances (Singh et al., 1992).

The ISWT comprises a 10 metre shuttle course with the walking speed externally paced or controlled using pre-recorded audio signals from a computerised disc. It consists of twelve incremental levels, with each level lasting one minute. It is a symptom limited test with the endpoint of the test determined when the patient becomes too breathless to continue or when the patient becomes unable to complete the shuttle in the paced time (defined as being 0.5metres or further from the marker). All patients were instructed to walk at a steady pace walk aiming to turn around at each end of the course at the sound of the audio signal. All were advised to continue to walk until they felt unable to maintain the required speed without becoming unduly breathless. The level achieved was recorded and total distance covered calculated. At the start and end of the ISWT all patients had their heart rate, oxygen saturations and dysnoea score according to the modified BORG dyspnoea scale (Borg, 1982) in accordance with standardised guidelines (2002).



#### **2.14 Health related quality of life**

Two health related quality of life (HRQL) questionnaires were used.

##### *St Georges Respiratory Questionnaire*

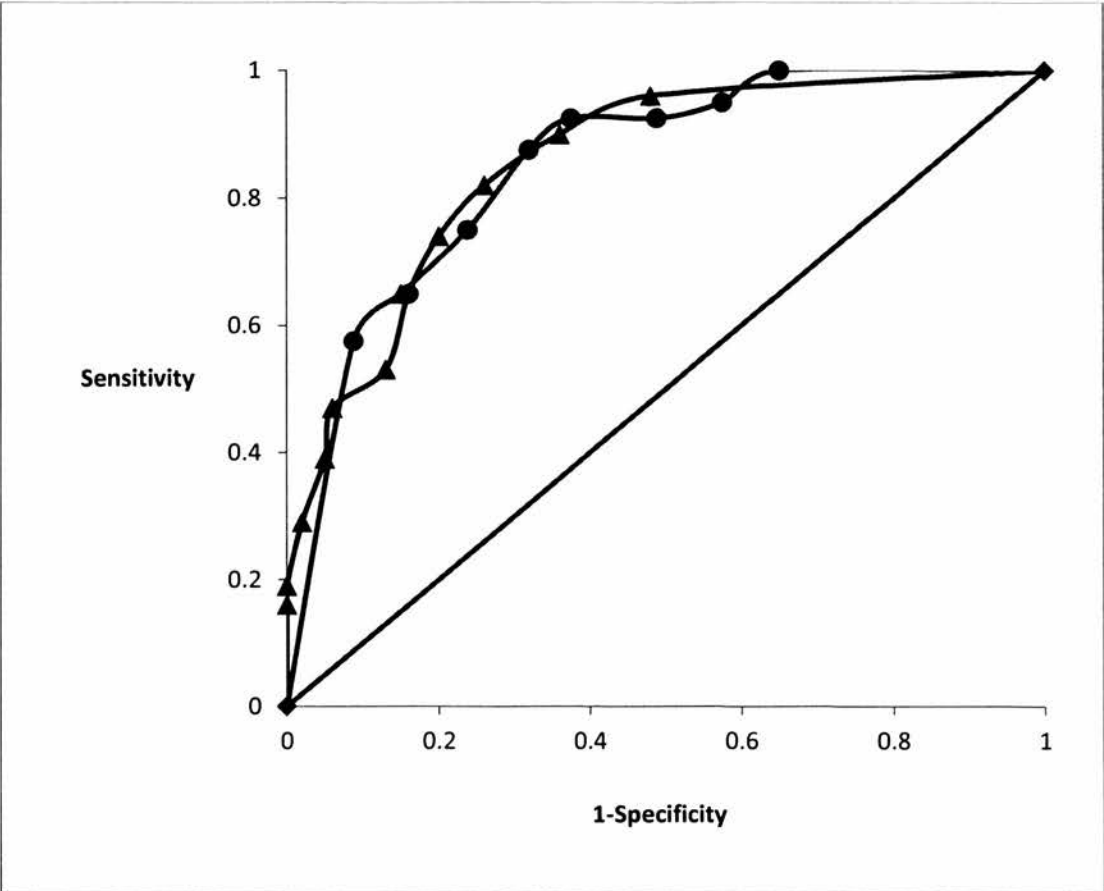
The St George's Respiratory Questionnaire is a 50 item self-administered health related quality of life questionnaire assessing the impact of multiple chronic respiratory symptoms on HRQL (Jones et al., 1992). It consists of 3 components- symptoms (8 items), activity (16 items) and impacts (26 items)- and has previously been validated to reflect impaired health related quality of life in bronchiectasis patients (Wilson et al., 1997a). The total score ranges from 0-100, with a higher score indicating a poorer health related quality of life. It assesses the impact of symptoms over the preceding four weeks. The minimum clinically important difference for change is 4 units (Wilson et al., 1997a).

### *Leicester Cough Questionnaire*

The Leicester Cough Questionnaire (LCQ) is a 19 item self-completed quality of life measure of chronic cough (Birring et al., 2003). It has 3 domains: physical (8 items), psychological (7 items) and social (4 items). The total severity score ranges from 3-21, with a lower score indicating greater impairment of health status due to cough. It assesses the impact of symptoms over the preceding two weeks.

The LCQ focuses purely on the impact of cough severity on HRQL rather than encompassing multiple respiratory symptoms and has been validated for use in bronchiectasis by exploring its reliability, responsiveness and validity in a two year prospective cohort study (Murray et al., 2009d). The ability of the LCQ to discriminate between mild and severe disease was explored in 120 patients - 51 with severe disease, 29 with moderate disease and 40 with mild disease. In predicting severe bronchiectasis, the LCQ was found to perform well, with an area under the receiver operator curve (AUC) score of 0.84 (0.80-0.88,  $P < 0.0001$ ) (Figure 9) and the lower the LCQ score the greater the likelihood ratio for severe disease (Table 7).

Figure 9. Predicting severe and mild bronchiectasis.



The utility of the LCQ in indicating severe and mild disease was assessed using the area under the receiver operator curve (AUC). For interpretation of AUC values the following is widely accepted: AUC 0.50-0.59= no value of test; 0.6-0.69= poor discrimination value; 0.70-0.79= moderate discriminatory value; 0.80-0.89= good discriminatory value; 0.9-1.0= excellent discriminatory value. ▲= severe ●=mild.

Table 7. The utility of the LCQ as a predictor of severe bronchiectasis.

<b>LCQ Score</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>LR+</b>	<b>LR-</b>	<b>Post- Test Odds Ratio</b>	<b>Positive Predictive Value</b>	<b>Negative Predictive Value</b>
<b>≤ 10</b>	29.4	98.5	19.6	0.7	14.5	93.7	65.4
<b>≤ 11</b>	39.2	95.6	8.9	0.6	6.6	87	68
<b>≤ 12</b>	47	94.2	8.1	0.5	6.0	85.7	71
<b>≤ 13</b>	52.9	86.9	4.0	0.5	3.0	75	71
<b>≤ 14</b>	64.7	84.0	4.0	0.4	3.0	75	76
<b>≤ 15</b>	74.5	79.7	3.7	0.3	2.7	73	81

The prevalence of severe disease in the study cohort of 120 patients was 42.5%. The pretest odds ratio was 0.74.

LR+ = the likelihood ratio for a positive test result and LR- = the likelihood ratio for a negative test result.

In predicting mild bronchiectasis, the LCQ performed well, with an AUC of 0.80 (0.76-0.84,  $P<0.0001$ ) (Figure 9) and the higher the LCQ score the greater the likelihood ratio for mild disease (Table 8).

Table 8. The utility of the LCQ as a predictor of mild bronchiectasis.

<b>LCQ Score</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>LR+</b>	<b>LR-</b>	<b>Post-Test Odds Ratio</b>	<b>Positive Predictive Value</b>	<b>Negative Predictive Value</b>
<b>≥ 20</b>	57.5	91.2	6.5	0.5	3.3	76.7	81.1
<b>≥ 19</b>	65	85	4.3	0.4	2.2	68.4	82.9
<b>≥ 18</b>	75	76.2	3.15	0.3	1.6	61.2	85.9
<b>≥ 17</b>	87.5	68.7	2.8	0.18	1.4	58.3	91.7

The prevalence of mild disease in the study cohort of 120 patients was 33.33%. The pretest odds ratio was 0.5.

LR+ = the likelihood ratio for a positive test result and LR- = the likelihood ratio for a negative test result.

The same prospective cohort study showed it to be a repeatable questionnaire in stable disease when completed by 67 patients over a six month period with an intraclass correlation of 0.96 (95% confidence interval 0.93-0.97) and a mean difference between the total scores of 0.1 (-0.4 to 1.0).

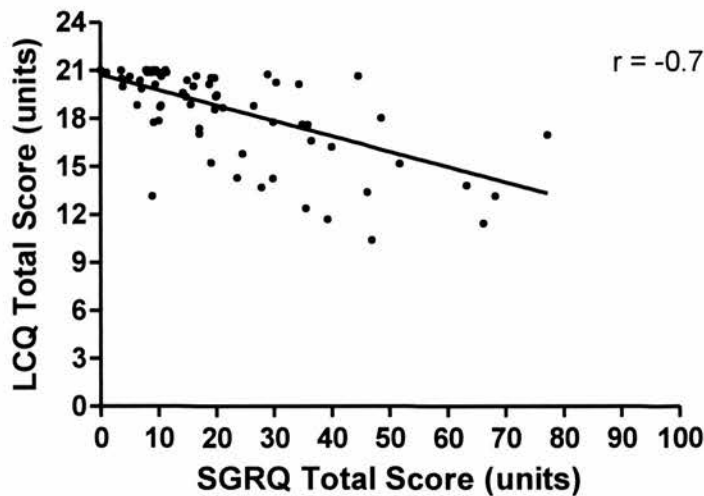
Importantly, the LCQ has been proven to be responsive to change and the minimum clinically important difference for change (MCID) has been shown to be 1.3units (Raj et al., 2009).

#### *SGRQ and LCQ*

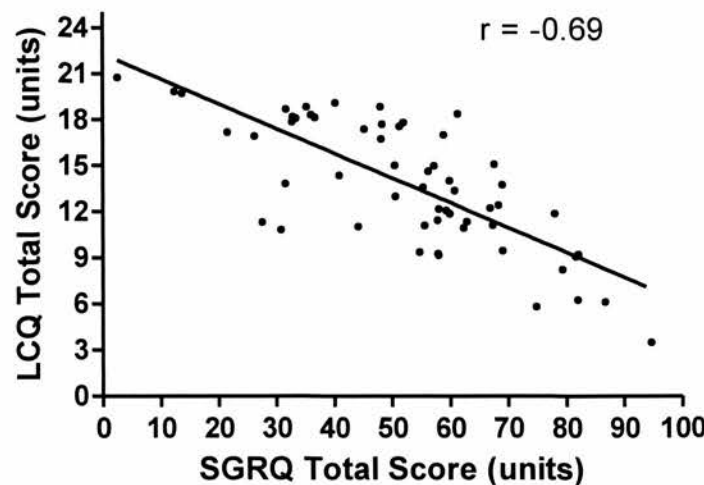
The LCQ has been shown to have a significant inverse correlation with the SGRQ in stable disease with a Spearman rank correlation coefficient was -0.7, (95% confidence interval -0.58 to -0.78,  $P<0.0001$ ). In exacerbations, the LCQ also has a significant inverse correlation with the SGRQ [Spearman rank correlation coefficient of -0.69 (95% confidence interval -0.53 to -0.81,  $P<0.0001$ ], Figure 10 (Murray et al., 2009d).

Figure 10. Correlation of the LCQ with the SGRQ in bronchiectasis.

**Correlation of the LCQ and the SGRQ total scores  
in stable bronchiectasis**



**Correlation of the LCQ and the SGRQ total scores  
in exacerbations**





### **2.15 Exacerbation Frequency**

An exacerbation was defined as a clinical deterioration with all of the following: increasing cough, increasing sputum volume and worsening sputum purulence (Anthonisen et al., 1987). All patients who experienced an exacerbation during the study were reviewed and received fourteen days of antibiotics (prescribed according to sputum bacteriology culture and sensitivities). All were reviewed following completion of antibiotics to ensure recovery. Patients were all supplied with a diary card for recording their exacerbations, particularly in case they were unable to attend for review. The diary card asked patients to document the following information: date of onset of symptoms; presence or absence of cough, sputum, chest pain, breathlessness or fever; the colour of the sputum according to the previously described colour chart (included within the diary); the date of commencement of any antibiotic treatment for their symptoms; the name of the antibiotic and duration of treatment; the actual date of feeling back to normal health. A copy of the diary card is enclosed (Appendix 9.2). Further specific instructions relevant to the individual studies are discussed in the individual studies' methods section.

**CHAPTER THREE:**

**A RANDOMISED CROSSOVER TRIAL OF CHEST PHYSIOTHERAPY IN**

**BRONCHIECTASIS**

### **3.1 Introduction**

Chest physiotherapy aims to mobilise bronchopulmonary secretions and facilitate effective expectoration, providing control of cough and improving airway clearance. It is widely advocated as a mainstay of management for bronchiectasis (O'Neill et al., 2002). To date however, there are no randomised controlled trials of chest physiotherapy exclusively in patients with non-cystic fibrosis bronchiectasis (Jones and Rowe, 2000).

Several different techniques or regimens for airway clearance exist such as postural drainage, autogenic drainage, the active cycle of breathing technique, positive expiratory pressure (PEP), oscillatory PEP devices and high frequency chest wall percussion. Previous small studies in non-cystic fibrosis bronchiectasis have compared various techniques and found no single method to be superior, although patient preference for technique has varied (Eaton et al., 2007, Thompson et al., 2002).

The aim of this randomised crossover study was to establish the efficacy of routine chest physiotherapy in non-cystic fibrosis bronchiectasis, comparing the effect of twice daily physiotherapy using an oscillatory PEP device with no chest physiotherapy in patients not previously practising regular chest physiotherapy.

### **3.2**     **Methods**

This was a randomised crossover trial of 3 months of twice daily chest physiotherapy followed by a one month washout period compared with 3 months of no chest physiotherapy in adults with non-cystic fibrosis bronchiectasis not routinely practising chest physiotherapy. Randomisation was determined by computer generation. The primary outcome was patient perceived cough severity measured using the Leicester Cough Questionnaire (LCQ) (Birring et al., 2003). Secondary outcomes included 24 hour sputum volume, FEV<sub>1</sub>, FVC, mid-expiratory flows (FEF<sub>25-75</sub>), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP), exercise capacity using the ISWT (Singh et al., 1992), sputum microbiology and the St George's Respiratory Questionnaire (Jones et al., 1992).

#### *Patients*

Patients were recruited according to the criteria described in the General Methods section. All patients were clinically stable at the point of entry to the study (defined as no change in routine respiratory medication and no requirements for antibiotics or corticosteroid therapy in the 4 weeks preceding the study entry date). Patients who were ex-smokers of less than two years or ex-smokers with a greater than ten pack year history of smoking and emphysema on high resolution CT scan were excluded.

#### *Physiotherapy*

Chest physiotherapy was carried out using the oscillatory PEP device “Acapella Choice®” (Smiths Medical ASD, Inc., NH03431, USA). Each patient was trained to complete 3 sets of the following cycle for each treatment session: 10 breaths (each

inhaling to three-quarters of the maximum inspiratory capacity then a 3 second breath hold followed by exhalation to functional residual capacity) and then 2-3 forced expiratory techniques (huffs) or coughs. The frequency/resistance dial (range 1-5) was set at 3 for all participants. This setting was the maximum tolerated by all participants. Patients completed 2 treatment sessions each day (morning and evening, typical duration 20-30 minutes). Compliance and occurrence of any adverse effects (specifically, any haemoptysis or increased use of short acting bronchodilator therapy) were assessed using a diary card which was reviewed monthly during the treatment phase. Technique was reviewed at monthly intervals during the treatment phase and the devices were retained at the Royal Infirmary of Edinburgh during the non-treatment phase.

#### *Other interventions*

*Exacerbations requiring antibiotic therapy:* exacerbations were diagnosed and managed as described in the General Methods section. No additional chest physiotherapy was advised.

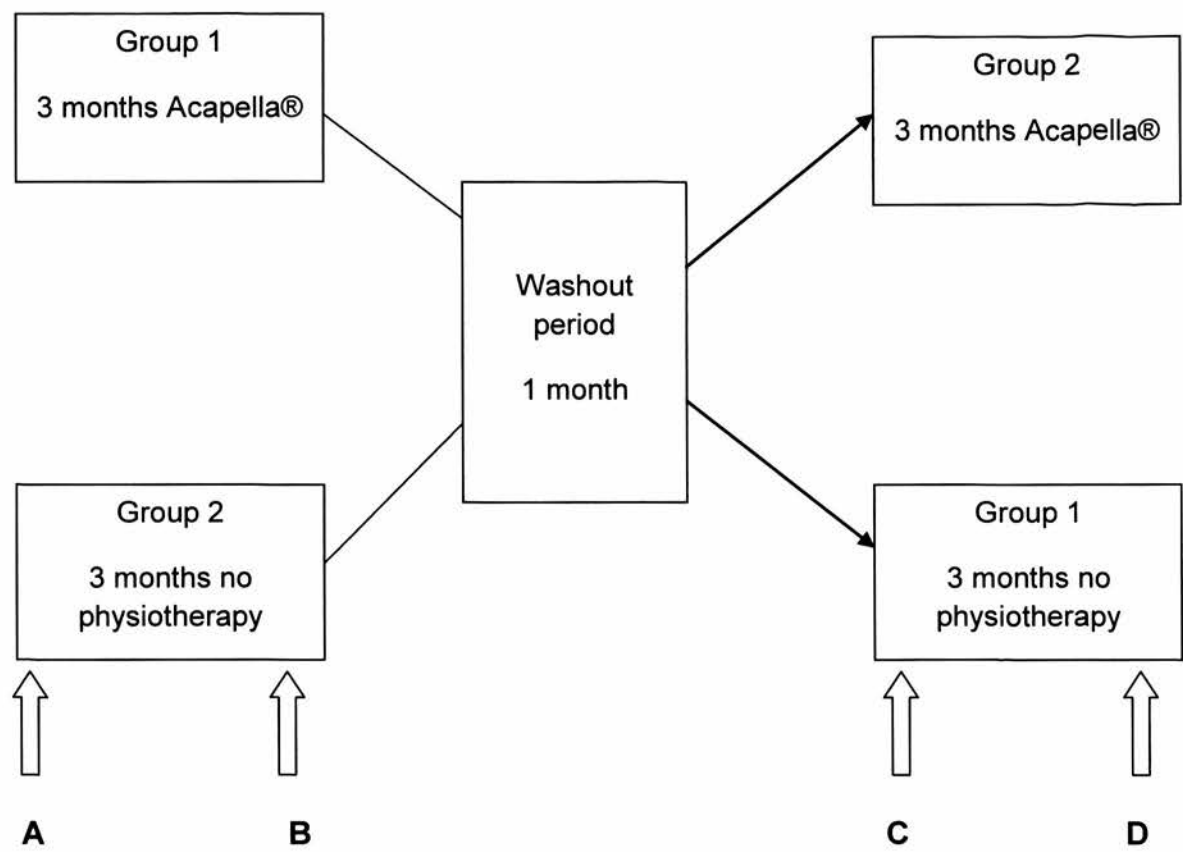
*Routine therapy:* any changes made to the patients' usual respiratory medication during the study period were noted.

#### *Endpoints*

The study design and assessment timepoints are shown in Figure 11. The following were assessed as described in the General Methods section at each timepoint: 24hr sputum volume; qualitative and quantitative sputum bacteriology; FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP and MEP; exercise capacity using the incremental shuttle walk test; health related

quality of life using the Leicester Cough Questionnaire and the St George's Respiratory Questionnaire.

Figure 11. Study protocol for a randomised crossover trial of physiotherapy in bronchiectasis.



Assessments were performed at the start and end of each treatment period with A, B, C and D representing the assessment time points.

### *Statistics*

Statistical analysis was performed using SPSS for Windows, Version 17 (SPSS inc, Illinois). The primary aim was to detect an improvement in LCQ score. Using the original validation of the LCQ, with 20 patients, a 5% level of significance (2 tailed), a common standard deviation of 0.94 and a power of 80% this would detect a mean difference in LCQ score of 0.63 (Birring et al., 2003). Using the validation of the LCQ in non-cystic fibrosis bronchiectasis, with 20 patients, a 5% level of significance (2 tailed), a common standard deviation of 1.1 and a power of 80% this would detect a mean difference in LCQ score of 0.73 (Murray et al., 2009d). Data was analysed according to the method of Kenward and Jones (Jones B, 1989). The treatment effect was estimated using the differences of the matched pairs of observations from each patient and is presented as median (interquartile range). Treatment differences between regular chest physiotherapy and no chest physiotherapy were compared using the Wilcoxon test. A 2-tailed P value of  $<0.05$  was considered significant.



### **3.3** **Results**

#### *Patients*

20 outpatients were recruited. Baseline demographics and patient characteristics are detailed in Table 9.

Table 9. Patients included in the randomised crossover study of chest physiotherapy.

<b>Characteristic</b>	<b>n(%) or median(IQR)</b>
<b>Study participants (all outpatients)</b>	20
<b>Male</b>	12(60)
<b>Age (years)</b>	73(72-77)
<b>Ex-smokers</b>	8(40)
<b>Chronic cardiac disease</b>	3(15)
<b>Neurological disease</b>	1(5)
<b>Chronic renal impairment</b>	1(5)
<b>Diabetes Mellitus</b>	0
<b>Inhaled corticosteroid therapy</b>	12(60)
<b>Systemic corticosteroid therapy</b>	0
<b>Long term antibiotic therapy</b>	2(10)
<b>Infective exacerbations requiring antibiotic treatment in preceding 12 months</b>	2(1.5-3)
<b>Lobes affected with bronchiectasis on HRCT</b>	4(3-4.75)
<b>Varicose or cystic dilatation affecting <math>\geq 1</math> lobe</b>	15(75)
<b>Chronically colonised with pathogenic organisms in sputum when stable</b>	14(70)
<i>Pseudomonas aeruginosa</i>	6(42.9)
<i>Haemophilus influenzae</i>	5(35.7)
<i>Staphylococcus aureus</i>	2(14.3)
<i>Moraxella catarrhalis</i>	1(7.1)
<b>Aetiology of bronchiectasis:</b>	
<b>Post-infective</b>	10 (50)
<b>Idiopathic</b>	8 (40)
<b>Inactive allergic bronchopulmonary aspergillosis</b>	1 (5)
<b>Inflammatory Bowel Disease</b>	1 (5)

*Study Entry- baseline and post-washout*

The baseline characteristics for the clinical and laboratory endpoints at entry to each phase of the study are detailed in Table 10. There was no significant difference in total LCQ score, 24 hour sputum volume, FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP, MEP, exercise capacity, sputum microbiology and SGRQ score (Table 10) between entry to the first arm of the study (Figure 10, Point 'A') and at the end of the washout period (Figure 10, Point 'C'), prior to entry to the second arm of the study.

Table 10. Comparison of clinical and laboratory assessments of patients at point of entry to each phase of the crossover study.

<b>Characteristic</b>	<b>Median(IQR) Study Start (Figure 1, Point 'A')</b>	<b>Median(IQR) End of Washout Period (Figure 1, Point 'C')</b>	<b>P</b>
<b>Total LCQ Score (units, range 3-21)</b>	16.3(14.1-17.9)	15.9(13.8-19.4)	0.5
<b>24hr sputum volume (ml)</b>	5(1.25-15)	5(1-13.1)	0.3
<b>FEV<sub>1</sub> (L) (% Predicted)</b>	1.68(1.25-2.31) 75.7(48.3-98.1)	1.72(1.19-2.10) 68.4(53-107.1)	0.8
<b>FVC (L) (% Predicted)</b>	2.64(1.9-3.65) 79.5(68.2-95.4)	2.82(1.75-3.5) 81.6(66.1-95.4)	0.6
<b>FEV<sub>1</sub>/FVC (% Predicted)</b>	0.63(0.57-0.77) 87.1(77.6-104.4)	0.62(0.56-0.82) 97.0(76.1-120)	0.7
<b>FEF<sub>25-75</sub> (L.s<sup>-1</sup>) (% Predicted)</b>	0.95(0.64-1.54) 47.5(21.9-64.8)	1.09(0.54-1.84) 44.8(24.8-96.4)	0.96
<b>MIP (cmH<sub>2</sub>O) (% Predicted)</b>	43.5(33.2-72.5) 58.5(37.2-77.2)	48(32.5-61.5) 51.7(31.1-63)	0.2
<b>MEP (cmH<sub>2</sub>O) (% Predicted)</b>	68.5(58.5-95.2) 60.9(41.9-83.4)	67(51-109) 51.3(38.3-55.7)	0.3
<b>Exercise capacity (m)</b>	220(120-405)	210(137.5-357.5)	1.0
<b>Sputum bacterial load (cfu.ml<sup>-1</sup>)</b>	3.8x10 <sup>6</sup> (3.9x10 <sup>5</sup> -3.8x10 <sup>7</sup> )	1.1x10 <sup>6</sup> (1x10 <sup>3</sup> -1.1x10 <sup>8</sup> )	0.6
<b>Total SGRQ Score (units, range 0-100)</b>	41.1(24.6-44.8)	40.4(18.0-52.5)	0.6

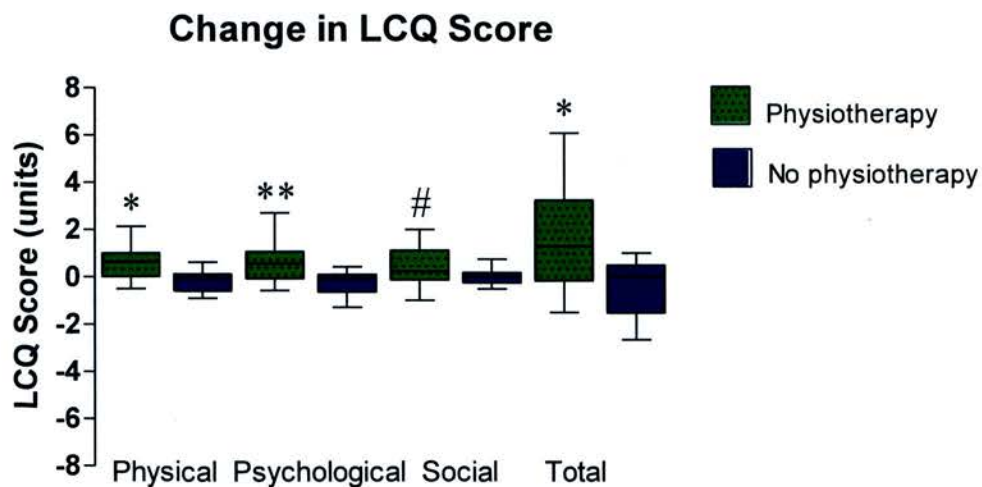
### *Completion and adverse events*

All patients completed the study and no adverse effects with the oscillatory PEP device occurred. There were 12 exacerbations affecting 11 patients during the study period (Table 11). No other interventions or changes to patients' care were required throughout the duration of the study.

### *Treatment differences*

There was a significant improvement in all of the domains and the total LCQ score (Table 11 and Figure 12).

Figure 12. Effect of regular chest physiotherapy on the LCQ.



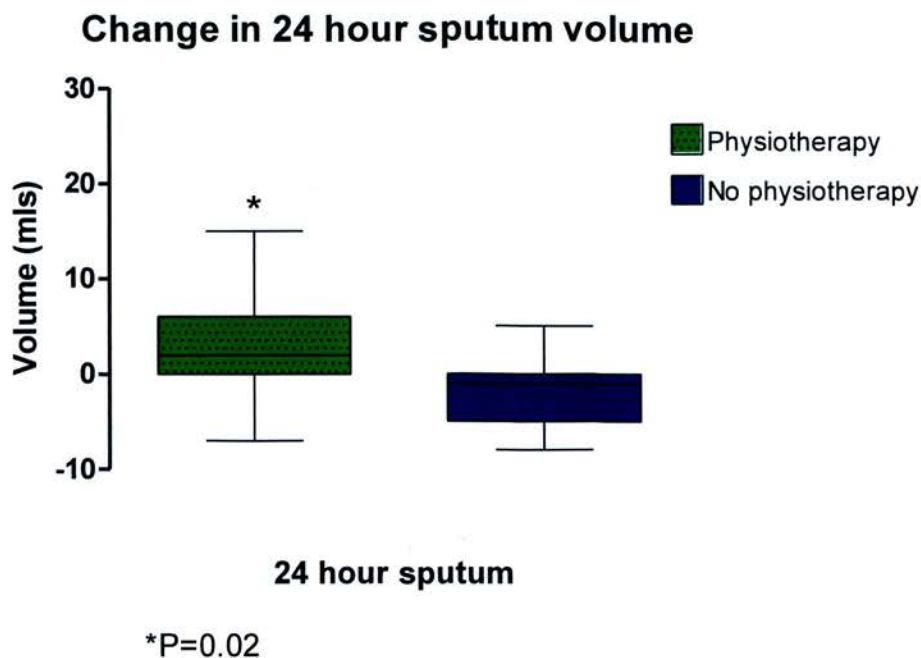
### **LCQ Domains and Total**

\*P=0.002 \*\*P<0.0001 #P=0.02

The change in LCQ individual domain and total scores with physiotherapy and with no physiotherapy is shown. The horizontal lines represent the median and interquartile ranges for each group and the whiskers represent the maximum and minimum. The median(IQR) score improved with regular chest physiotherapy from 4.5(4.1-5.8)units to 5.1(4.9-6.2)units in the Physical domain; from 5.1(4.6-6.1)units to 6.3(5.4-6.7)units in the Psychological domain; from 5.7(4.2-6.1)units to 6.2(5.0-7.0)units in the Social domain and from 15.3(13.5-17.9) to 18.2(15.4-19.4) in the Total score. \*P=0.002 \*\*P<0.0001 #P=0.02.

24 hour sputum volume significantly increased with regular chest physiotherapy compared with no chest physiotherapy (Figure 13).

Figure 13. Effect of regular chest physiotherapy on 24 hour sputum volume.

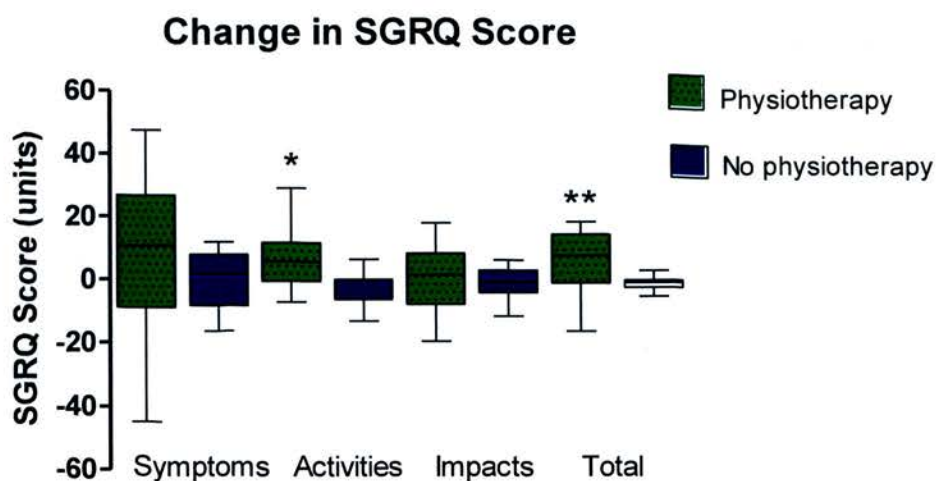


The change in 24 hour sputum volume with physiotherapy and with no physiotherapy is shown. The horizontal lines represent the median and interquartile ranges and the whiskers represent the maximum and minimum. \*P=0.02.

With regular chest physiotherapy, the median (IQR) 24 hour sputum volume increased from 5(2-15)mls to 12.5(2.5-20)mls.

The total SGRQ score improved significantly with regular chest physiotherapy but the only significant improvement seen in the individual domains of the SGRQ score was in the activity domain (Figure 14).

Figure 14. Effect of regular chest physiotherapy on the SGRQ.



#### SGRQ Domains and Total

\*P=0.02 \*\*P=0.005

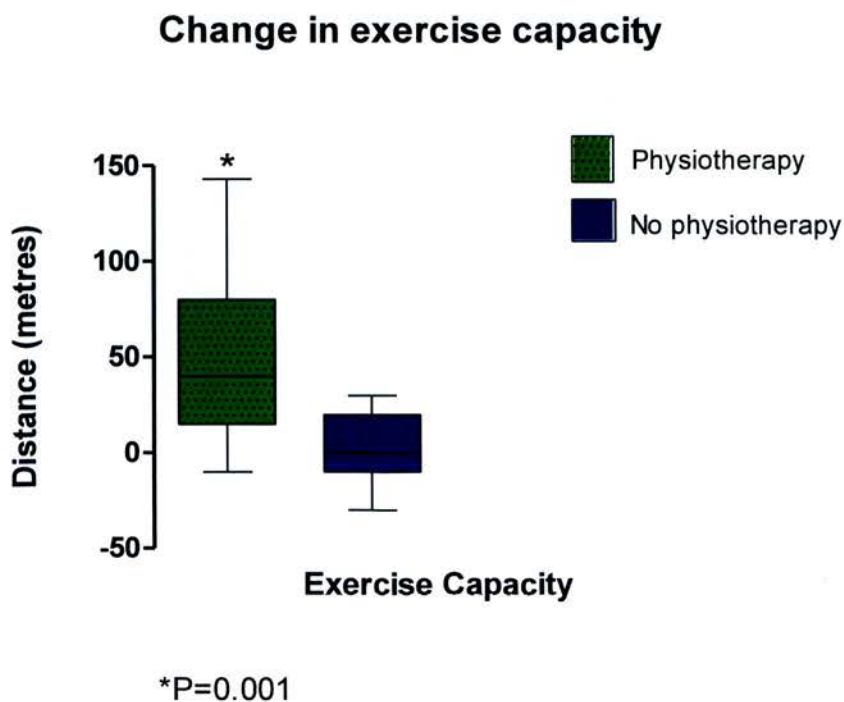
The change in SGRQ individual domain and total scores with physiotherapy and with no physiotherapy is shown. The horizontal lines represent the median and interquartile ranges for each group and the whiskers represent the maximum and minimum. \*P=0.02 \*\*P=0.005.

Regular chest physiotherapy significantly improved the Activities domain score from 47.6(32.6-59.4)units to 47.3(17.4-59.4)units and the Total score from 41.1(24.6-49.1)units to 32.8(13.9-41.0)units. There were no statistically significant improvements seen in the other domains, with the Symptoms score improving from 50.8(33.5-74)units to 42.8(27.2-53.3)units and the Impacts score improving from 30.2(15.5-41.5)units to 23.9(7.2-28.7)units.



Exercise capacity also improved significantly with regular chest physiotherapy (Figure 15) but there were no significant differences seen in sputum bacteriology, FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP or MEP, or exacerbation frequency (Table 11).

Figure 15. Effect of regular chest physiotherapy on exercise capacity.



The change in exercise capacity with physiotherapy and with no physiotherapy is shown. The horizontal lines represent the median and interquartile ranges for each group and the whiskers represent the maximum and minimum. \*P=0.001.

Exercise capacity improved from 220(125-355)metres to 330(200-430)metres with regular chest physiotherapy.

Table 11. Treatment differences with regular physiotherapy and no physiotherapy.

<b>Outcome</b>	<b>Twice daily physiotherapy Median(IQR)</b>	<b>No regular physiotherapy Median(IQR)</b>	<b>P</b>
<b>Total LCQ Score Improvement (units)</b>	1.3(-0.17-3.25)	0(-1.5-0.5)	0.002
<b>24hr sputum volume (ml)</b>	2(0-6)	-1(-5-0)	0.02
<b>FEV<sub>1</sub> (L)</b>	-0.01(-0.06-0.08)	-0.01(-0.1-0.11)	0.7
<b>FVC (L)</b>	-0.01(-0.09-0.28)	0.06(-0.08-0.21)	0.9
<b>FEF<sub>25-75</sub> (L.s<sup>-1</sup>)</b>	-0.02(-0.17-0.16)	0.04(-0.1-0.34)	0.6
<b>MIP (cmH<sub>2</sub>O)</b>	-1(-9-7)	5.5(-10-12.5)	0.7
<b>MEP (cmH<sub>2</sub>O)</b>	5(-11-25)	8.5(-3.7-19.7)	0.7
<b>Exercise capacity (m)</b>	40(15-80)	0(-10-20)	0.001
<b>Sputum bacterial load (cfu.ml<sup>-1</sup>)</b>	-1x10 <sup>3</sup> (-2.78x10 <sup>6</sup> - 1.74x10 <sup>7</sup> )	1x10 <sup>3</sup> (-6.5x10 <sup>7</sup> -6.4x10 <sup>6</sup> )	0.72
<b>Total SGRQ Score Improvement (units)</b>	7.8(-0.99-14.5)	-0.7(-2.3-0.05)	0.005
<b>Number of exacerbations</b>	5	7	0.48

### **3.4 Discussion**

This randomised crossover trial found that twice daily chest physiotherapy in patients with non-cystic fibrosis bronchiectasis not normally practising regular physiotherapy, significantly improves perceived cough severity, increases 24 hour sputum volume, improves exercise capacity and SGRQ score, but has no effect on sputum microbiology, FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP, MEP or exacerbation frequency.

Clearance of bronchopulmonary secretions is impaired in patients with bronchiectasis (Currie et al., 1987b). Mazzocco et al (Mazzocco et al., 1985) first explored potential benefits of chest physiotherapy in bronchiectasis patients over 2 decades ago but despite further studies investigating various physiotherapy techniques to aid clearance, there have been no randomised controlled studies exploring the efficacy of regular chest physiotherapy. The primary outcome measured was selected to reflect one of the major goals of management of chronic disease- an improvement in HRQL. Specifically, the aim was to assess the impact of the predominant symptom of bronchiectasis- cough severity.

The study population had clinically significant bronchiectasis. They had an average of 2(1.5-3) exacerbations in the preceding 12 months, over two-thirds were chronically colonised with pathogenic organisms in their sputum and radiologically, 4(3-4.75)lobes were affected with bronchiectasis on CT chest scan and 75% had varicose or cystic dilatation in at least one lobe. Despite this, they did not carry out regular chest physiotherapy prior to the study.

The study was conducted with twice daily chest physiotherapy over 3 months, as an outpatient. The Acapella Choice® device was selected as the airway clearance

technique both for ease of use and based on patient preference from previous studies (Thompson et al., 2002, Currie et al., 1986). Currently, there is no clear evidence for the optimum frequency or duration of airway clearance. However, it is recognised that airway clearance regimens need to be effective without affecting other activities of daily living. A twice daily physiotherapy regimen was selected to account for this and as previous studies assessing different physiotherapy techniques for non-cystic fibrosis bronchiectasis have achieved compliance with this frequency (Thompson et al., 2002, Patterson et al., 2007). Further studies are needed to specifically address the optimal frequency and duration of physiotherapy.

Several adjuncts to physiotherapy currently exist including: bronchodilator therapy which may minimise bronchial hyperactivity and improve airway clearance; inhaled hyperosmolar agents (nebulised hypertonic saline has been shown to yield greater sputum weights and inhaled mannitol which has been shown in small studies to date to aid mucociliary clearance (Kellett et al., 2005, Daviskas et al., 2008)); pulmonary rehabilitation and inspiratory muscle training has previously been shown to improve exercise tolerance (Newall et al., 2005). Inhaled mucolytics (recombinant human DNase) on the other hand, although of benefit in cystic fibrosis, is not recommended in non-cystic fibrosis bronchiectasis because it has a significant negative impact on FEV<sub>1</sub> (O'Donnell et al., 1998). This study did not employ any such adjuncts. Further studies are needed to assess efficacy of such techniques in addition to regular chest physiotherapy.

This study did not have a sham arm and instead twice daily chest physiotherapy using an oscillatory PEP device was compared with no chest physiotherapy. This design was

intentional as any type of sham would involve some form of airway clearance. A limitation of this study design, however, is the potential for a placebo effect. The crossover design was used to offer all patients a period of regular chest physiotherapy during the study. There was no carry over effect with the 1 month washout phase.

One of the main goals of long term management of chronic respiratory disease is to improve HRQL and with regular, controlled airway clearance, patient perception of cough severity improved with an increase in score seen in all domains of the LCQ. Since the completion of this study, a 1.3 unit difference in the total LCQ score has been established as a clinically significant change (Raj et al., 2009) and a median increase of 1.3 units following 3 months of twice daily chest physiotherapy was indeed observed in this study. A previous open label study by Mutalithas et al found a mean improvement of 3.1 units in total LCQ score with bronchopulmonary hygiene physical therapy (Mutalithas et al., 2008). Importantly, although this current study was not powered to detect a change in SGRQ score, this also improved, with the total score improving beyond its established minimal clinically significant change of 4 units (median improvement of 7.8 units) (Wilson et al., 1997a).

A major rationale for chest physiotherapy is to loosen secretions and enhance expectoration. According to previous work by Cecins et al, this study was sufficiently powered to detect a 15% change in 24hour sputum volume and we found that with twice daily chest physiotherapy the mean volume of sputum expectorated over 24 hours increased by 2(0-6)ml (Cecins et al., 1999). Although the value of 24 hour sputum collections in clinically stable outpatients may be limited by patient compliance and confounded by factors such as swallowed secretions, it is a highly pertinent, non-

invasive marker and has been selected as a relevant outcome measure in previous studies assessing other potential long term therapeutic strategies in bronchiectasis including inhaled steroids and long term antibiotics (Tsang et al., 2005a, Tsang et al., 1999b, el-Din et al., 1994, Cymbala et al., 2005).

An increase in the distance achieved during the ISWT following 3 months of twice daily chest physiotherapy was also observed. Based on a previous study by Newall et al the patient sample size was powerful enough to detect a significant effect on exercise capacity (Newall et al., 2005). This perhaps emphasises the improvement in the activities domain of the SGRQ and that with greater control of cough and clearance of mucus from the airways, exercise capacity also improves.

The benefits observed with regular chest physiotherapy were small however, and we did not find any improvements in the remaining study endpoints. Despite increased sputum expectoration, we observed no change in sputum bacterial load or any improvement in FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP, MEP or exacerbation frequency. One previous study of airway clearance techniques in bronchiectasis comparing the Flutter device with the active cycle of breathing technique found a significant improvement in FEV<sub>1</sub> (0.08L, 95% Confidence Interval 0.01 to 0.15 using the Flutter device), however, this improvement in FEV<sub>1</sub> was not thought to be clinically significant (Thompson et al., 2002). The patient cohort in the current study had a median age of 73(72-77)years and with increasing age, there is less airway reversibility, perhaps further limiting the opportunity for any benefit from chest physiotherapy on FEV<sub>1</sub> or FVC. In addition, there were no improvements in the assessment of small airways or inspiratory and expiratory pressures. This may however, be due to the intrinsic variability of such

measurements. A previous study by Newall et al in non-cystic fibrosis bronchiectasis patients found MEPs to be impaired but MIPs were within normal range (Newall et al., 2005). The current study population was however, older than that of Newall's study which may explain the difference. Despite the lack of improvements in these measures, exercise capacity significantly increased.

In conclusion, this randomised crossover study found that regular chest physiotherapy in non-cystic fibrosis bronchiectasis has significant benefits compared with no chest physiotherapy. Despite the differences being small, achieving an improvement in functional ability and HRQL is highly relevant to the management of this long-term illness. Larger studies are needed to explore potential benefits on other outcome measures.

The next study was designed to assess the impact of reducing bacterial infection in the airways on both clinical and laboratory outcomes during acute exacerbations of bronchiectasis to assess whether infection and inflammation can be reduced and clinical improvement achieved.

**CHAPTER FOUR:**  
**ASSESSING RESPONSE TO TREATMENT OF EXACERBATIONS IN**  
**BRONCHIECTASIS**



#### **4.1 Introduction**

Infective exacerbations are a significant cause of morbidity in non-cystic fibrosis bronchiectasis and often necessitate utilisation of health care resources including inpatient admissions for intravenous antibiotic therapy (Kelly et al., 2003). The criteria used to define exacerbations requiring antibiotic therapy have been described in chronic obstructive pulmonary disease and include increasing dyspnoea, increasing sputum production and worsening sputum purulence (Anthonisen et al., 1987). Similarly, in bronchiectasis, antibiotics are prescribed in exacerbations in patients with increasing cough, increasing sputum volume and worsening sputum purulence. Little is known about how best to assess the effect of antibiotic treatment in the management of such exacerbations. Currently, a successful outcome is qualitative only, relying on the patient's subjective assessment of symptom resolution. Validated, easily accessible and relevant outcome measures are needed (Chang and Bilton, 2008).

It is not known if the outcome of an exacerbation is influenced by the pathogenic organism. It has been shown that patients who are chronically colonised with *Pseudomonas aeruginosa* have more severe bronchiectasis, a poorer health related quality of life and may have an accelerated decline in FEV<sub>1</sub> (Martinez-Garcia et al., 2007, Evans et al., 1996, Wilson et al., 1997b, Ho et al., 1998).

The aim of this prospective cohort study was to assess the effect of two weeks intravenous antibiotic therapy for exacerbations of non-cystic fibrosis bronchiectasis on a variety of both clinical and laboratory endpoints including 24 hour sputum volume, FEV<sub>1</sub>, FVC, exercise capacity, systemic inflammation, sputum microbiology and health

related quality of life. In addition, the aim was to determine whether these outcomes were influenced by the pathogenic organism isolated.

## **4.2   Methods**

This was a prospective cohort study of adults with infective exacerbations of non-cystic fibrosis bronchiectasis requiring intravenous antibiotics.

Patients with an exacerbation were selected according to the criteria described in the General Methods. Patients with mixed normal flora isolated from their sputum at the start of the exacerbation were excluded from the study. Criteria for intravenous antibiotic therapy included failure of oral antibiotics, culture of pathogenic organisms sensitive only to intravenous agents or severe exacerbations necessitating acute inpatient admission.

All patients received 14 days of intravenous antibiotic therapy; the antibiotics chosen were based on sputum microbiology prior to commencing treatment or, if necessary, previous sputum microbiology history. All were advised to continue their routine respiratory medications and chest physiotherapy. Any patient on long term oral corticosteroid therapy had their dose doubled for the first seven days of the exacerbation. No other adjunctive treatments were used to manage the exacerbation.

The following endpoints were assessed as described in the General Methods at the start of the exacerbation prior to commencement of treatment (Day 0) and on completion of antibiotics (Day 14): 24hr sputum volume; qualitative sputum bacteriology; FEV<sub>1</sub> and FVC; exercise capacity using the incremental shuttle walk test (ISWT); total leukocyte count (WCC); erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); St

George's Respiratory Questionnaire Score (SGRQ). The SGRQ was completed at the start of the exacerbation and one week following antibiotic completion. The questionnaire was adapted for the end of exacerbation assessment to ask about symptoms in the preceding week (i.e. the single week following antibiotic completion). This version of the SGRQ has not been externally validated.

### *Statistics*

Statistical analysis was performed using SPSS for Windows, Version 16 (SPSS inc, Illinois) and GraphPad Prism Version 5.0 (GraphPad Software, California). The data were normally distributed and is presented as mean  $\pm$  standard deviation. For comparing means, the paired t-test was used. Patients that isolated *Pseudomonas aeruginosa* were compared with those that isolated other potential pathogenic microorganisms (other PPMs) at the start of the exacerbation. Fishers exact test was used to compare groups. A 2 tailed P value of  $<0.05$  was considered to be statistically significant. Additional sub analysis was conducted to ensure that the reason for needing intravenous therapy or those who went on to experience multiple exacerbations did not influence the results.

### **4.3   Results**

#### *Patients*

There were 58 exacerbations of non-cystic fibrosis bronchiectasis managed with 2 weeks of intravenous antibiotic therapy during the study period. 13 patients had more than 1 exacerbation during the study period, but only the first exacerbation for each patient was used. Five patients were excluded as mixed normal flora was isolated at the start of the exacerbation and one patient was excluded as they had mucoid sputum at presentation. The final analysis was conducted in 32 individual patients.

Table 12 details baseline patient characteristics, including parameters of disease severity, and table 13 provides details of the aetiology of the bronchiectasis. All patients had advanced bronchiectasis with 29 patients (90.6%) being chronically colonised with pathogenic bacteria in the sputum when clinically stable (65.6% had *Pseudomonas aeruginosa*) and having multiple exacerbations in the past year (Table 12). 62.5% needed intravenous antibiotics because of failure of oral antibiotics, 34.4% had severe exacerbations necessitating acute inpatient admission and 3.1% required intravenous antibiotics as the pathogenic organisms isolated were sensitive only to agents available as intravenous preparations.

Table 12. Clinical characteristics of patients included in the study of intravenous antibiotics for exacerbations of bronchiectasis.

<b>Characteristic</b>	<b>n (%) or Mean <math>\pm</math> Standard Deviation</b>
<b>Number</b>	32
<b>Male</b>	10 (31.3)
<b>Age (years)</b>	62.8 $\pm$ 9.8
<b>Non smokers</b>	23 (71.9)
<b>Ex-smokers</b>	9 (28.1)
<b>Ex-smoker pack year history</b>	34.5 $\pm$ 17.2
<b>COPD</b>	3 (9.4)
<b>Asthma</b>	18 (56.3)
<b>Inhaled Corticosteroid Therapy</b>	28 (87.5)
<b>Nebulised Bronchodilator Therapy</b>	7 (21.9)
<b>FEV<sub>1</sub> (L)</b>	1.57 $\pm$ 0.58
<b>(% Predicted)</b>	(66.5 $\pm$ 27.3)
<b>FVC (L)</b>	2.49 $\pm$ 0.78
<b>(% Predicted)</b>	(85.3 $\pm$ 33.2)
<b>Infective exacerbations in preceding year</b>	7.4 $\pm$ 6.0
<b>Number of lobes involved on HRCT chest</b>	4.1 $\pm$ 1.7
<b>Cystic bronchiectasis</b>	10 (31.2)
<b>Chronically colonised</b>	29 (90.6)

Table 13. Aetiology of bronchiectasis of patients included in the study of intravenous antibiotics for exacerbations of bronchiectasis.

<b>Aetiology</b>	<b>n (%)</b>
Inactive allergic bronchopulmonary aspergillosis	11 (34.4)
Burnt out sarcoidosis	1 (3.1)
Post Infective (Pneumonia, Tuberculosis, Pertussis, Measles)	10 (31.3)
Immunoglobulin Deficiency (all IgG subclass2)	4 (12.5)
Primary ciliary dyskinesia	1 (3.1)
Idiopathic	5 (15.6)

### *Sputum Bacteriology*

At the start of the exacerbation *Pseudomonas aeruginosa* was isolated in 19 patients; 10 had non-mucoid and 9 had mucoid *Pseudomonas aeruginosa*. 13 patients isolated other PPMs which included *Haemophilus influenzae* (n=4), *Streptococcus pneumoniae* (n=3), *Staphylococcus aureus* (n=2), *Moraxella catarrhalis* (n=2), *Escherichia coli* (n=1) and *Serratia* species (n=1).

### *Antibiotic Therapy*

All patients completed 14 days of antibiotic therapy and doses used were as recommended for bronchiectasis/cystic fibrosis in British National Formulary (JointFormularyCommittee., 2008). In the *Pseudomonas aeruginosa* group 13 received intravenous ceftazidime (2g, three times daily) and gentamicin (dose per ideal body weight and creatinine clearance); 2 patients with a previous history of gentamicin toxicity received intravenous ceftazidime (2g, three times daily) and colistin (2MU three times daily) and three with known impaired renal function received intravenous ceftazidime (2g, twice daily) and oral ciprofloxacin (500mg twice daily). The remaining patient who had a known intolerance of ceftazidime received intravenous piperacillin with tazobactam (4.5g, three times daily) and oral ciprofloxacin (500mg twice daily).

In the patients that isolated other PPMs, those who isolated *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* all received single agent ceftriaxone (2g once daily). 2 patients isolated *Staphylococcus aureus* (one methicillin sensitive *Staphylococcus aureus* and one methicillin resistant *Staphylococcus aureus*) and received intravenous flucoxacillin (500mg, four times daily) and vancomycin (dose per ideal body weight and creatinine clearance) respectively. The remaining two

patients isolated *Serratia* species and *Escherichia coli* respectively and both were managed with intravenous ceftazidime (2g, three times daily) and gentamicin (dose per ideal body weight and creatinine clearance).

#### *Corticosteroids*

4 patients were receiving long term oral corticosteroid therapy and had their dose doubled for 7 days. No patients otherwise received oral corticosteroid therapy.

#### *Side effects*

No adverse effects were reported and no changes to treatment regimens occurred and all patients successfully completed 14 days treatment.

#### *Outcomes: clinical assessments*

##### *24 hour sputum volume*

Following 14 days' antibiotics all patients' 24 hour sputum volume reduced with a mean reduction of  $21.8 \pm 19.9$  ml ( $P < 0.0001$ ) (Table 14).

All patients had a reduction in their 24 hour sputum volume: 5% had  $<25\%$  improvement, 15% had 25-49% reduction and 80% had  $\geq 50\%$  reduction in 24 hour sputum volume (Figure 16).



### *Pulmonary function tests*

The FEV<sub>1</sub> did not significantly improve, with a mean increase of 0.07±0.2L (5.4±12.5%), P=0.07 (Table 14). The FVC did however, improve by a mean of 0.09±0.44L (2.9±22.2%), P = 0.01 (Table 14).

29% of patients did not have any improvement in FEV<sub>1</sub>, 36% had an improvement of <10%, 19% had an improvement of 11-17% and 16% improved their FEV<sub>1</sub> by ≥ 18% (Figure 16).

### *Exercise capacity*

The distance achieved in the ISWT significantly improved by 58.3±51.0m (48.9±54.8%), P<0.0001 (Table 14) following 2 weeks' antibiotic treatment.

12% of patients did not show an improvement in exercise capacity, 28% had <25% improvement, 28% had <50% improvement and 36% of patients had an improvement of ≥50% (Figure 16).

### *Outcomes: laboratory assessments*

#### *Systemic inflammation*

There was a significant improvement in WCC, ESR and CRP (Table 14). WCC reduced by a mean of 3.7±3.7 x10<sup>9</sup>/L, ESR reduced by a mean of 17.7±21mm/hr and CRP reduced by a mean of 59.5±69.6mg/L (Table 14).

WCC did not improve in 9.7% of patients, 29% had 50-74% improvement and no patients had a ≥75% improvement in WCC (Figure 16).

ESR did not improve in 30% of patients, 40% had a 50-74% improvement and 7% had a  $\geq 75\%$  improvement in ESR (Figure 16).

CRP did not improve in 9.3% of patients, 15.6% had a 50-74% improvement and 62.5% had a  $\geq 75\%$  improvement in CRP (Figure 16).

### *Sputum microbiology*

Qualitative bacterial clearance was achieved in 78.1% of patients ( $P < 0.0001$ ) (Figure 15). All patients infected with other PPMs achieved bacterial clearance. 12 out of 19 patients infected with *Pseudomonas aeruginosa* achieved bacterial clearance. Of the 7 with *Pseudomonas aeruginosa* who did not achieve bacterial clearance, 5 of these had a mucoid strain.

### *Health related quality of life*

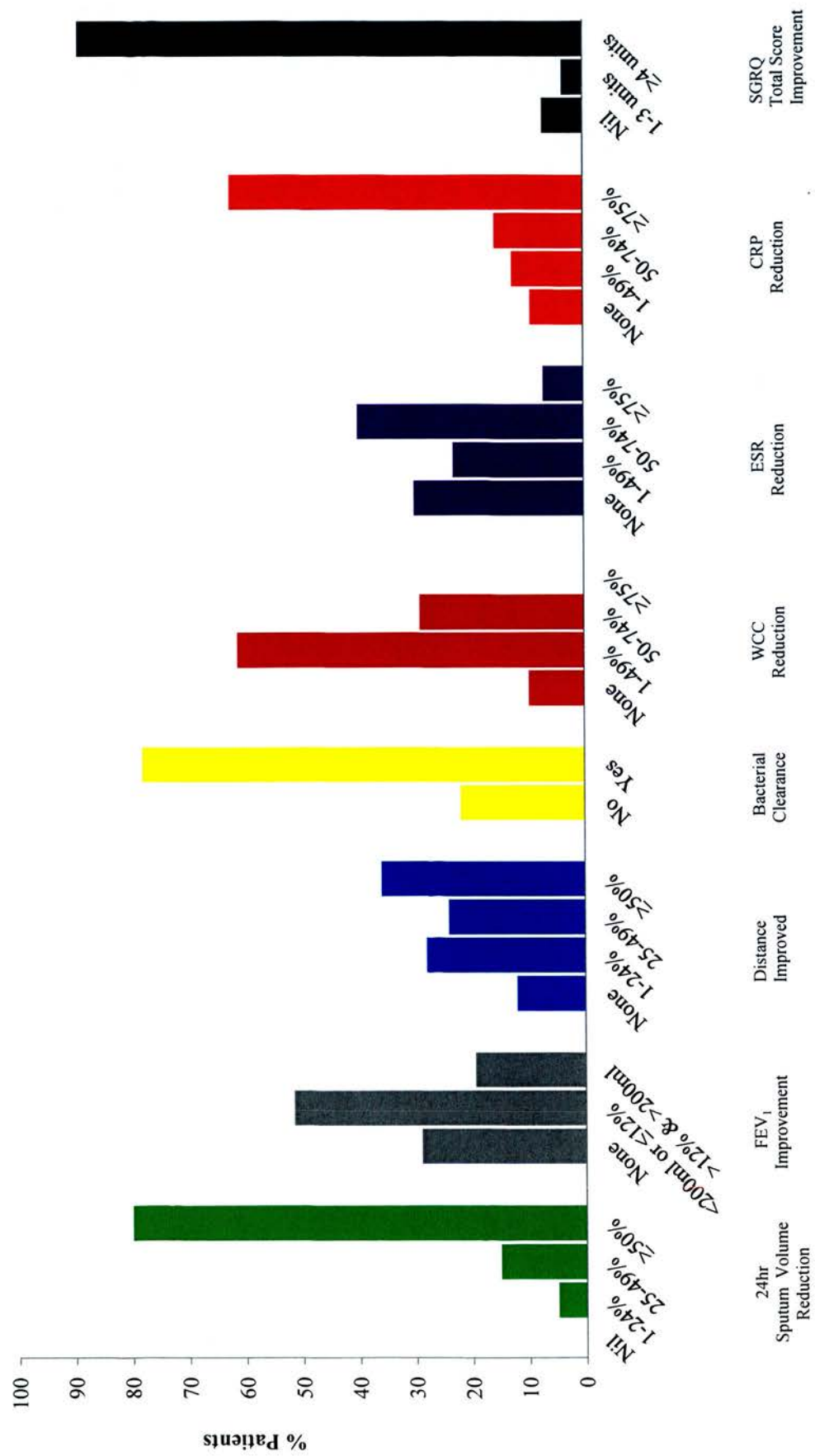
The SGRQ total score significantly improved with a mean reduction of  $13.8 \pm 12.9$  units ( $P = 0.01$ ) after completion of antibiotics (Table 14).

7.1% did not show any improvement in SGRQ score, 3.6% had an improvement of  $< 4$  units and 89.3% had an improvement of  $> 4$  units.

Table 14. Outcome measures at the start and the end of the exacerbation.

Variable	Start of exacerbation	End of exacerbation	P
24 hr sputum volume (mls)	30.4 ± 21.9	8.5 ± 8.4	<0.0001
FEV <sub>1</sub> (L)	1.45 ± 0.57	1.52 ± 0.58	0.07
FVC (L)	2.35 ± 0.78	2.5 ± 0.86	0.01
FEV <sub>1</sub> /FVC (L)	61.4 ± 12.3	60.4 ± 11.5	0.5
Exercise Capacity (m)	217.0 ± 168.0	271 ± 184.0	<0.0001
WCC (x10 <sup>9</sup> /L) (normal range 4-11)	10.8 ± 7.1	7.2 ± 2.5	<0.0001
CRP (mg/L)	66.9 ± 70.7	7.4 ± 11.2	<0.0001
ESR (mm/hr)	40.1 ± 27.4	22.3 ± 14.1	<0.0001
SGRQ score	57.9 ± 17.5	45.7 ± 20.0	<0.0001

Figure 16: Changes in clinical markers following 2 weeks of intravenous antibiotic therapy in exacerbations of bronchiectasis.



Observed parameter & response

### *Pathogenic organism*

The response to treatment was compared between patients with PA and other PPM. The responses were not significantly different with the exception of FEV<sub>1</sub> and FVC. Patients infected with *Pseudomonas aeruginosa* showed no improvement in either FEV<sub>1</sub> (mean value at start of exacerbation was 1.52±0.61 improving to only 1.53±0.58 at the end, P=0.9) or in FVC (mean value at start of exacerbation 2.38 ± 0.88 improving to 2.44 ± 0.81L, P=0.3). Those patients infected with other PPMs did however, have a significant improvement in both FEV<sub>1</sub> (improving from 1.32±0.48L to 1.51±0.61, P=0.01) and FVC (improving from 2.25±0.62L to 2.58±0.96L, P=0.02).

### *Need for Intravenous Antibiotics*

11 patients required intravenous antibiotics because of failure of oral antibiotics, 1 patient due to culture of pathogenic organisms sensitive only to intravenous agents and 20 patients due to severe exacerbations necessitating acute inpatient admission.

At the start of the exacerbation, the need for hospitalisation group had worse exercise capacity (mean difference -196 ± 59.3m, p = 0.003), higher CRP (mean difference 49.8 ± 23.4mg/L, p = 0.04) and white cell count (mean difference 2.8 ± 1.3 × 10<sup>9</sup>, p = 0.04) and worse SGRQ score (mean difference 14.4 ± 5.8 units, p = 0.02). There was no significant difference in the other endpoints.

Both groups showed a significant improvement in all parameters except in FEV<sub>1</sub> and FVC.

### *Single versus multiple exacerbations*

19 patients had one exacerbation necessitating intravenous antibiotics and 13 required more than one course of intravenous antibiotics ( $3.6 \pm 1.5$  courses). At the start of the exacerbation there was no difference in the endpoints between groups. Patients with a single exacerbation had a significant improvement in all parameters except in FEV<sub>1</sub> and FVC. Patients with more than one exacerbation had a significant improvement in all parameters except FEV<sub>1</sub>.

### **4.3 Discussion**

This prospective cohort study identified useful clinical and laboratory markers for assessing the response to 2 weeks' intravenous antibiotic treatment in adults with exacerbations of non-cystic fibrosis bronchiectasis. Improvements were observed in 24 hour sputum volume, sputum bacterial clearance, systemic inflammation, exercise capacity and health related quality of life, independent of pathogenic organism. No improvement was observed in FEV<sub>1</sub>. 24 hour sputum volume, microbial clearance, CRP and SGRQ were the most responsive markers identified.

All patients received 14 days of intravenous antibiotic therapy. The optimum duration of treatment is not known and further trials will be needed to define this period but previous studies have administered therapy for between 5 to 28 days (Darley et al., 2000, Chan et al., 1996, Ip et al., 1993, Tsang et al., 1999a).

All patients with *Pseudomonas aeruginosa* or Gram negative organisms were treated with two anti-pseudomonal antibiotics to reduce the chance of developing antibiotic resistance. Those with other PPMs were treated with monotherapy using intravenous ceftriaxone. The optimum therapeutic agents are unknown. The agents used in this study are known from in vitro sensitivities to be effective agents. The aim of the study was not to assess the therapeutic regimen but to explore the outcome of two weeks' effective antibiotic therapy on a variety of clinical and laboratory parameters.

There are no published international guidelines and no validated methods to allow clinicians to assess response to treatment. Currently, a successful outcome relies predominantly on the subjective assessment of symptom resolution. Objective outcome

measures are needed (Chang and Bilton, 2008). This study sought to observe relevant, minimally invasive and easily accessible endpoints to assess parameters that may be clinically useful in monitoring response to therapy. Four particularly responsive markers were identified: 24 hour sputum volume, qualitative sputum bacteriology, CRP and SGRQ score.

All patients had a significant improvement in 24 hour sputum volume, with 80% having a  $\geq 50\%$  reduction. 24 hour sputum volume is a potentially useful marker as it is highly pertinent to the condition, non-invasive, easily accessible and inexpensive. It has been used as a marker in previous studies that have assessed potential long term therapeutic strategies including inhaled steroids and long term antibiotics (Tsang et al., 2005a, Cymbala et al., 2005, el-Din et al., 1994, Currie et al., 1990). A recent study has shown wet weight sputum to be as reliable dry sputum (Moran F, 2006). The potential limitation of 24 hour sputum volume as an outcome measure is the reliance on patient compliance for collection.

Sputum bacteriology as an endpoint could involve either complete bacterial clearance or a reduction in bacterial load (colony forming units/ml). Direct counts of bacterial load is time consuming, expensive and less likely to be available in the majority of routine (non research) microbiology laboratories. In this study 78.1% had bacterial clearance. In the remaining 22.9% there is no information on whether there was a reduction in bacterial load as only qualitative cultures were carried out. However, these patients did show improvements in the 3 other key parameters identified in this study: 24 hour sputum volume (mean reduction  $13.8 \pm 9.1$  mls), CRP (mean reduction  $61.4 \pm 104.4$  mg/ml) and SGRQ score (mean reduction  $14.8 \pm 7.2$  units). Further studies using both qualitative and



quantitative cultures would be helpful, although at present, the findings emphasise the need for more than one endpoint to assess response to treatment.

A 9 fold fall in C-reactive protein (mean $\pm$ SD fall 59.5 $\pm$ 69.6mg/L) was observed, with 62.5% having a  $\geq$ 75% improvement following antibiotic therapy. The other markers of systemic inflammation observed were WCC and ESR. Although a significant improvement was seen in WCC (1.6 fold fall with a mean $\pm$ SD fall of 4.0 $\pm$ 4.3  $\times 10^9$ /L), the mean WCC at the start of the exacerbation was within normal range and the reduction following antibiotics was small, suggesting WCC is a less relevant marker of treatment response. The mean ESR also improved (1.8 fold fall with a mean $\pm$ SD fall of 17.7 $\pm$ 20.9mm/hr) however, only 7% of patients achieved  $\geq$ 75% reduction in ESR.

89.7% of patients showed a clinically significant response (i.e.  $\geq$ 4 unit improvement) in SGRQ score. The use of such questionnaires remains predominantly within the research setting as administration and analysis require specific resources and are time consuming.

In keeping with the observed clinical improvements, exercise capacity improved by 1.2 fold with a mean $\pm$ SD rise of 54.1 $\pm$ 51.5m and 36% of patients increasing their distance in the ISWT by  $\geq$  50%. It may be a useful adjunct to other markers.

There was no significant improvement in FEV<sub>1</sub> but there was a small 1.06 fold rise in FVC (mean $\pm$ SD rise of 144 $\pm$ 306ml). There was however, an improvement seen in FEV<sub>1</sub> in patients infected with other PPMs which may reflect the complete bacterial clearance achieved in this group. A recent review article of exacerbations in bronchiectasis noted FEV<sub>1</sub> to be a less sensitive measure in acute exacerbations, particularly in contrast to

cystic fibrosis bronchiectasis (Chang and Bilton, 2008). Similar findings were made by the authors of a previous study who found that following 2 weeks of treatment with oral amoxicillin there were small improvements in FEV<sub>1</sub> (mean improvement 8ml) and FVC (mean improvement 180ml) but these changes were not felt to be clinically useful (Hill and Stockley, 1986).

This study has identified multiple parameters which may be used to assess response to antibiotics in adults with exacerbations of bronchiectasis, independent of the pathogenic organism. 24 hour sputum volume, microbial clearance, CRP and SGRQ were most responsive to change.

The next study was designed to assess whether limiting the bacterial burden in the airways on a long-term basis can reduce airways inflammation, disrupting the vicious cycle of inflammation and infection in the airways thus improving clinical status and symptoms. A randomised controlled trial of nebulised antibiotics administered over a 12 month period was therefore conducted.

**CHAPTER FIVE:**  
**A RANDOMISED CONTROLLED TRIAL OF NEBULISED GENTAMICIN IN**  
**BRONCHIECTASIS**

## **5.1**   **Introduction**

Long term antibiotics offer a real therapeutic option in bronchiectasis: reducing the bacterial burden in the airways may reduce inflammation and promote healing of the bronchial tree, limiting symptoms and potentially improving health related quality of life. Targeted delivery of aerosolized antibiotics directly to the airways may have significant benefit with minimal adverse systemic effects.

The purpose of this randomised controlled trial was to assess the efficacy of continuous nebulised gentamicin therapy over one year in patients with non-cystic fibrosis bronchiectasis chronically infected with a variety of pathogens and to assess whether treatment effects are sustained over a three month treatment free follow up period. The primary endpoint was a greater than or equal to one log unit reduction in sputum bacterial load, regarded as the minimum important reduction necessary to have a significant impact on airways inflammation (Angrill et al., 2001).

## **5.2**   **Methods**

This was a randomised controlled trial of 12 months twice daily nebulised gentamicin compared with twice daily nebulised 0.9% saline, followed by a 3 month treatment-free follow-up period in adults with non-cystic fibrosis bronchiectasis. The primary outcome was  $\geq 1$  log unit reduction in sputum bacterial density. Secondary outcomes included qualitative sputum bacteriology, emergence of gentamicin resistant *Pseudomonas* strains, sputum myeloperoxidase and free neutrophil elastase, 24hr sputum volume, sputum purulence, FEV<sub>1</sub>, FVC, midexpiratory flows (FEF<sub>25-75</sub>), exercise capacity, the Leicester Cough Questionnaire (LCQ) and St George's Respiratory Questionnaire (SGRQ), exacerbations and side effects.

### *Patients*

Patients were recruited according to the criteria described in the General Methods section. All patients were clinically stable at the point of entry to the study (defined as no change in routine respiratory medication and no requirements for antibiotics or corticosteroid therapy in the 4 weeks preceding the study entry date). Patients who were current smokers or ex-smokers of less than one year or ex-smokers with a greater than twenty pack year history of smoking were excluded. In addition, patients had to be able to tolerate nebulised gentamicin (defined as no bronchospasm or fall in FEV<sub>1</sub> >15% and >200mls following nebulisation of 80mg gentamicin with or without prebronchodilation using nebulised 2.5mg salbutamol). Furthermore, patients with any of the following were excluded: creatinine clearance <30mls/minute; vestibular instability; previous documented intolerance to aminoglycosides (ototoxicity or nephrotoxicity).

### *Interventions*

*Study treatment:* randomisation was to either twice daily nebulised 80mg gentamicin (supplied as gentamicin injectable solution and reconstituted for nebulisation using 0.9% saline) or twice daily nebulised 5mls 0.9% saline. Porta-Neb Ventstream (Profile Respiratory Systems Ltd, Bognor Regis, West Sussex) nebulisers were used for both groups.

*Nebulised gentamicin treatment:* a regime of 80mg nebulised gentamicin twice daily was prescribed.

*Nebulised saline:* twice daily 5mls 0.9% saline was administered.

Any patient with a serum gentamicin level  $>1\text{mg/ml}$  had their study treatment reduced to once daily. Any patient reporting symptomatic bronchospasm (defined as increased wheeze and/or breathlessness) had adjunctive treatment with a nebulised  $\beta_2$  agonist (salbutamol 2.5mg) prior to their study treatment.

*Study compliance:* the twice daily treatment was self-administered at home. At each 3 monthly review, all patients were supplied with their study treatment as well as the appropriate number of needles, syringes and filters necessary for nebulisation for the following three months. They were asked to return any unused equipment or medication. This was counted and in addition, all were questioned directly regarding their compliance at each visit.

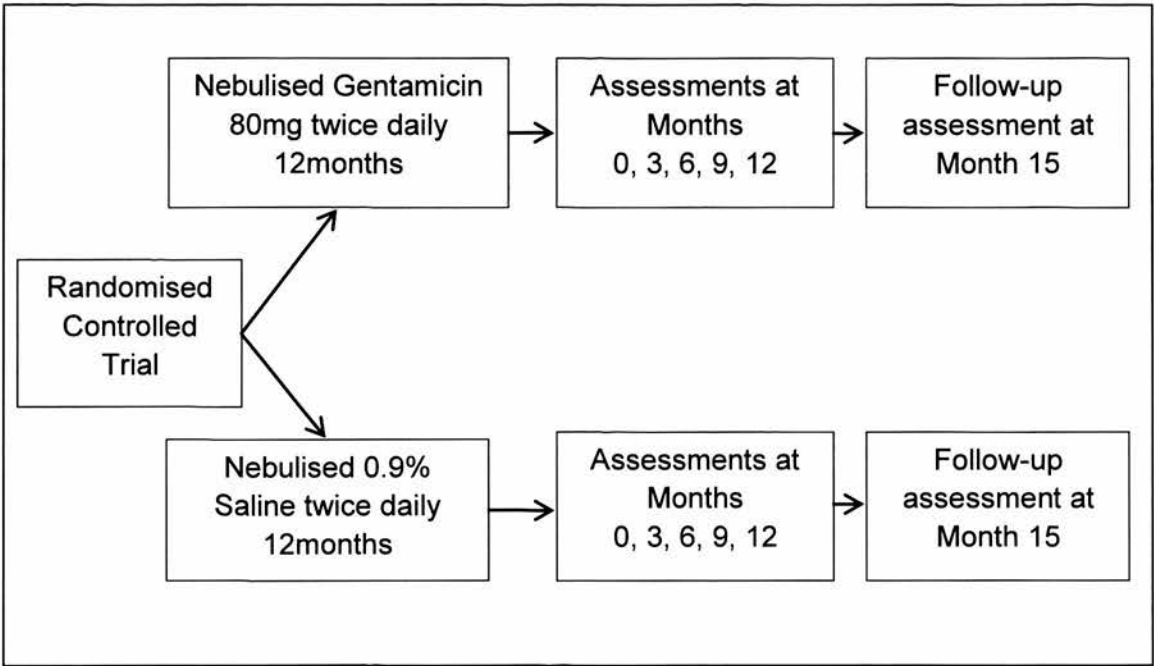
*Exacerbations requiring antibiotic therapy:* exacerbations were diagnosed and managed as described in the General Methods. Regular study treatment continued during the exacerbation.

*Routine therapy:* any changes made to the patients' usual respiratory medication during the study period were noted.

#### Assessments

All patients were reviewed at baseline entry to the study (month 0) and at months 3, 6, 9, 12 of treatment and then after a 3 month treatment free, follow-up period (month15),  
Figure 17.

Figure 17. Design of study of nebulised gentamicin versus nebulised saline in bronchiectasis.





At each review, the following assessments were conducted as described in the General Methods: sputum colour; 24hr sputum volume; quantitative and qualitative sputum bacteriology; sputum myeloperoxidase and elastase; total leucocyte count; erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); FEV<sub>1</sub>, FVC and FEF<sub>25-75</sub>; exercise capacity using the incremental shuttle walk test (ISWT); health related quality of life using the LCQ and the SGRQ.

In addition, gentamicin susceptibility testing was performed for all isolates of *Pseudomonas aeruginosa* and Gram negative enteric bacteria at months 0, 12 and 15 in the routine NHS laboratory, according to the Clinical and Laboratory Standards Institute Guidelines (2006). The following zone diameters were used with 10µg gentamicin disk to report susceptibility with *Pseudomonas* strains: resistance ≤12mm; indeterminate 13-14mm; sensitive ≥15mm (2006).

### *Toxicity*

Serum gentamicin levels as well as urea and electrolytes were measured at two weeks following initiation of treatment and then at each three monthly assessment until completion of the study and 3 months after completion of treatment. At the same time-points, all patients were directly asked whether they had any dizziness or change to their hearing.

## Statistics

All statistics were performed using SPSS for Windows, Version 17 (SPSS inc, Illinois) and GraphPad Prism Version 5.0 (Graphpad Software, La Jolla, California).

The primary aim was to detect a one log unit reduction in bacterial density (cfu/ml). Using a 2 sided, 2 sample test with 80% power and 1% level of significance, 24 patients per group were necessary and to allow for a 20% dropout rate we selected 30 patients per group.

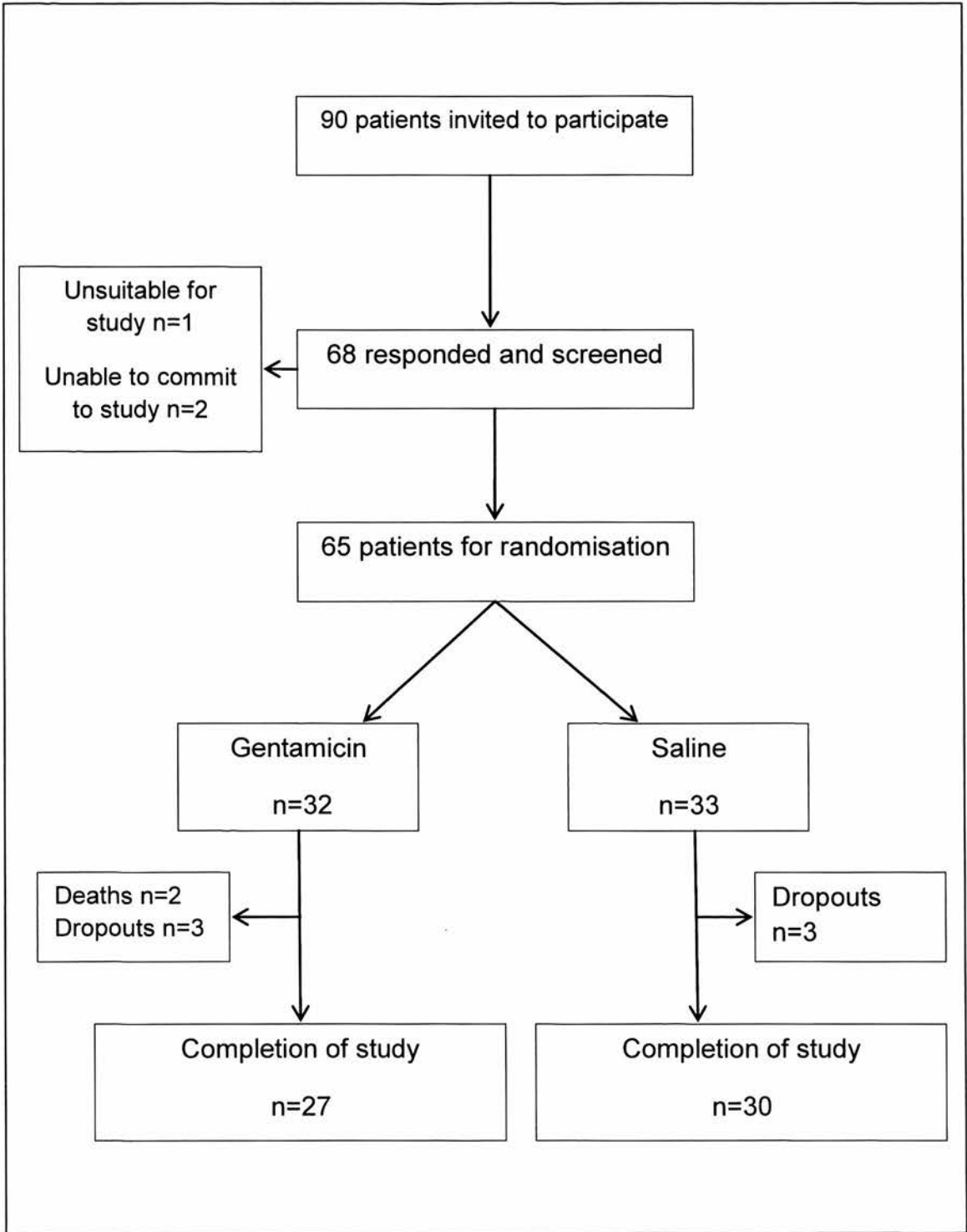
Only patients who completed the study were included in the final analysis. Analysis of the results comprised a Mann-Whitney U test for unpaired numerical data and Wilcoxon for paired numerical data were used. A Fishers exact test was used to compare categorical variables. Data are presented as median (interquartile range). A two-tailed P value of  $<0.05$  was considered statistically significant.

### **5.3**   **Results**

#### *Patients*

65 patients were recruited to the study, with 32 randomised to nebulised gentamicin and 33 to nebulised saline. In the gentamicin group, there were two unexpected sudden deaths (previously undiagnosed metastatic colorectal cancer at month three and myocardial infarction at month 5). There were no deaths in the saline group. In the gentamicin group, there were three withdrawals: two felt unable to tolerate the nebulised therapy (month 3 and month 6 respectively) and one felt unable to commit to the study reviews (month 6). In the saline group, there were three withdrawals, two of whom felt unable to tolerate the nebulised therapy (month 3 for both) and the third was unable to commit to the study reviews (month 3). In total, 57 patients completed the study, with 27 in the gentamicin group and 30 in the saline group (Figure 18).

Figure 18. Consort diagram showing patient recruitment for nebulised gentamicin versus nebulised saline in bronchiectasis.



The baseline characteristics for each group in the analysis are shown in Table 15.

Table 15. Patient characteristics at entry to study of nebulised gentamicin versus nebulised saline in bronchiectasis.

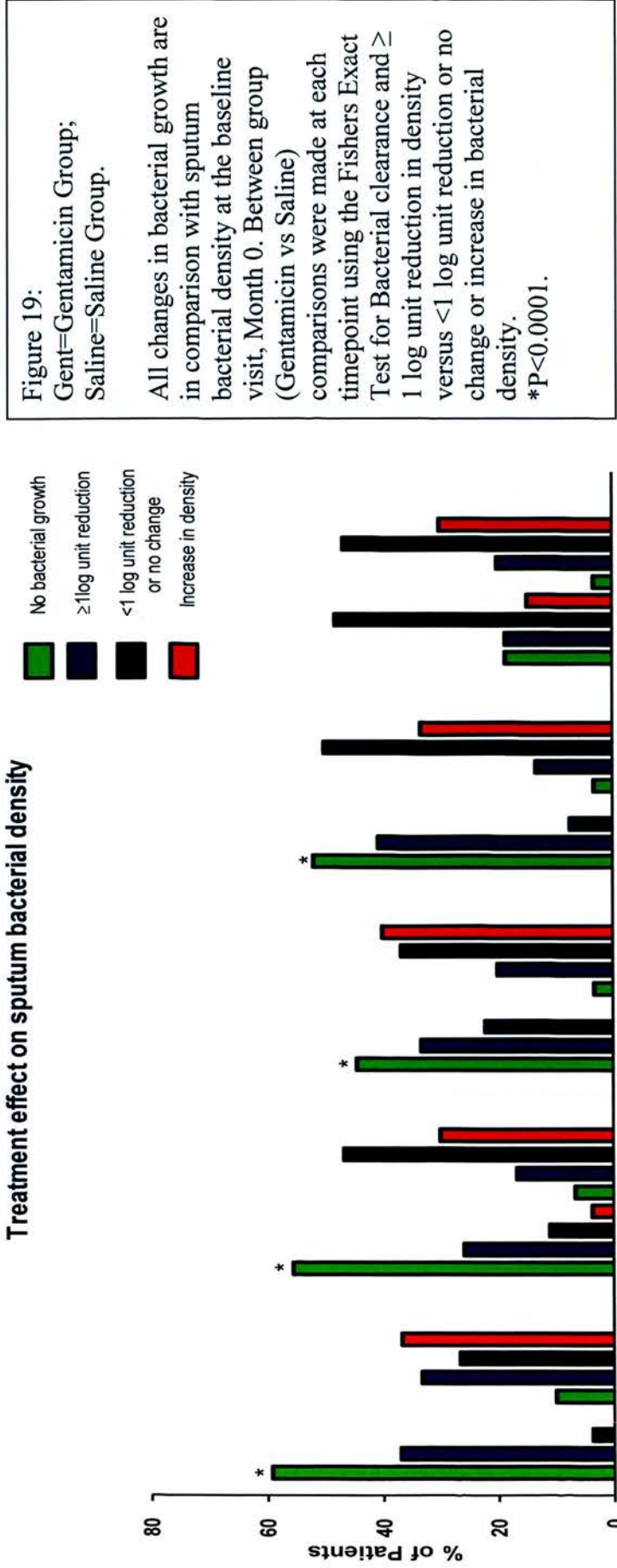
<b>Characteristic</b>	<b>Gentamicin Group n=27 n(%) or median(IQR)</b>	<b>Saline Group n=30 n(%) or median(IQR)</b>	<b>P</b>
<b>Male Sex</b>	9(33.3)	15(50)	0.2
<b>Age (years)</b>	58(53-67)	64(55.7-69)	0.2
<b>Ex-smokers</b>	8(29.6)	8(26.7)	0.8
<b>Pack Year History</b>	10(1.2-16)	7(4.2-16)	0.8
<b>Inhaled Steroid Therapy</b>	17(62.9)	19(63.3)	0.6
<b>Oral Corticosteroids</b>	3(11.1)	3(10)	0.9
<b>Inhaled <math>\beta_2</math> agonists</b>			
<b>Short acting</b>	19(70.4)	22(73.3)	0.8
<b>Long acting</b>	17(62.9)	19(63.3)	1.0
<b>Inhaled anticholinergics</b>			
<b>Short acting</b>	3(11.1)	0(0)	0.3
<b>Long acting</b>	1(3.7)	1(3.3)	0.2
<b>Aetiology</b>			
<b>-Idiopathic</b>	8(29.6)	9(30)	1.0
<b>-Post-infective</b>	11(40.7)	11(36.7)	0.7
<b>-Inactive ABPA</b>	6(22.2)	6(20)	0.8
<b>-Inactive Sarcoid</b>	1(3.7)	1(3.3)	0.9
<b>-Immunodeficiency (IgG<sub>2</sub> deficiency)</b>	1(3.7)	1(3.3)	0.9
<b>-Inflammatory Bowel Disease</b>	0	2(6.6)	0.2
<b>CT Characteristics</b>			
<b>-Cystic or varicose dilatation</b>	25(92.6)	22(73.3)	0.06
<b>-Lobes involved</b>	5(3-6)	5(3-6)	0.8
<b>-Reiff Severity Score</b>	9(5-12)	7.5(4-14)	0.6

## *Sputum bacteriology*

### Quantitative bacteriology

There was no significant difference in bacterial density between the groups at baseline with a median of 8.02(7.63-8.3)log<sub>10</sub>cfu/ml in the gentamicin group and 7.88(7.34-8.17)log<sub>10</sub>cfu/ml in the saline group, P=0.29. At the end of 12 months' treatment, the bacterial density had significantly reduced in the gentamicin group [2.96(1.0-5.9)log<sub>10</sub>cfu/ml] compared with the saline group [7.67(7.34-8.17)log<sub>10</sub>cfu/ml], P<0.0001. At follow up however, bacterial density was similar in both groups [gentamicin group 7.29(5.88-7.76)log<sub>10</sub>cfu/ml and saline group 7.49(6.46-8.19)log<sub>10</sub>cfu/ml, P=0.12]. Figure 19 shows the changes in bacterial density at each of the study timepoints for both groups. For each assessment timepoint, the impact of nebulised gentamicin and nebulised saline on sputum bacterial density compared with baseline bacterial density for each group is shown. The impact is categorised as: no bacterial growth;  $\geq 1$  log unit reduction in bacterial density;  $< 1$  log unit reduction or no change in bacterial density; an increase in bacterial density. At each assessment timepoint, the difference between each impact category was compared between the gentamicin and saline group using the Fishers exact test. There was a significant difference in impact between the gentamicin and saline groups at 3 months, 6 months, 9 months and 12 months. At month 15, there was no significant difference in the change seen between the two groups.

Figure 19. Effect of nebulised gentamicin and nebulised saline on sputum bacterial density.



\* $P<0.0001$ .

### Qualitative bacteriology

There was no significant difference in the infecting pathogen between the groups at baseline (Table 16a). Table 16b shows the change in sputum infection for both groups throughout the study.

By one year, within the gentamicin group, 30.8% (4 of 13 patients) infected with *Pseudomonas aeruginosa* achieved eradication and 3.7% (1 of 13 patients) cultured a different pathogen, *Staphylococcus aureus*. 92.8% (13 of 14 patients) originally infected with pathogens other than *Pseudomonas aeruginosa* achieved eradication. In those not achieving eradication, there was still a significant reduction in bacterial density from a median(IQR) 8.06(7.5-8.4)log<sub>10</sub>cfu/ml at baseline to 6.17(5.81-7.15)log<sub>10</sub>cfu/ml at the end of treatment.

At entry to the study, all patients with *Pseudomonas aeruginosa* and Gram negative enteric pathogens had strains fully susceptible to gentamicin. Of these, no patients in either group at the end of treatment or at follow up had developed gentamicin indeterminately resistant or resistant strains.



Table 16a. Sputum qualitative bacteriology prior to commencement of nebulised gentamicin and nebulised saline.

Timepoint	Gentamicin Group n(%)	Saline Group n(%)	P
<b>Baseline</b>			
Chronically infected	27(100)	30(100)	
- <i>Pseudomonas aeruginosa</i>	13(48.1)	11(36.7)	0.5
- <i>Haemophilus influenzae</i>	11(40.7)	15(50)	0.3
- <i>Staphylococcus aureus</i>	2(7.4)	1(3.3)	0.5
- <i>Streptococcus pneumoniae</i>	1(3.7)	0(0)	0.3
- <i>Moraxella catarrhalis</i>	0(0)	2(6.7)	0.5
-Other (coliforms)	0(0)	1(3.3)	0.3

Table 16b. Comparison of nebulised gentamicin and nebulised saline on sputum qualitative bacteriology throughout the study.

	Month 0 n(%)	Month 3 n(%)	Month 6 n(%)	Month 9 n(%)	Month 12 n(%)	Month 15 n(%)
<b>Gentamicin Group</b>						
<i>Pseudomonas aeruginosa</i> & Enteric Gram negative species	13(48.1)	8(29.6)	7(25.9)	8(29.6)	7(25.9)	10(37)
-Other PPMs	14(51.8)	4(14.8)	3(11.1)	3(11.1)	2(7.4) <sup>#</sup>	8(29.7)
-MNF or No Bacterial Growth	0	15(55.6)	17(62.1)	16(59.2)	18(66.7)	9(33.3)
<b>Saline Group</b>						
<i>Pseudomonas aeruginosa</i> & Enteric Gram negative species	12(40)	11(36.7)	10(33.3)	9(30)	9(30)	7(23.3)
-Other PPMs	18(60)	17(56.7)	19(63.3)	20(66.7)	20(66.7)	22(73.3)
-MNF or No Bacterial Growth	0	2(6.7)	1(3.3)	1(3.3)	1(3.3)	1(3.3)

Definition of abbreviations: PPMs= Potentially Pathogenic Microorganisms

(*H.influenzae*, *S.pneumoniae*, *M.catarrhalis*, *S.aureus*); MNF= mixed normal flora. #

3.7% (1 patient) was originally infected with *Pseudomonas aeruginosa* at entry to the study.

#### *Sputum neutrophil myeloperoxidase and free elastase*

There was no significant difference in sputum myeloperoxidase or free elastase activity at baseline between the two groups. At the end of treatment, concentrations of myeloperoxidase and free elastase activity were significantly lower in the gentamicin group compared with the saline group. At follow up however, concentrations of both myeloperoxidase and free elastase activity was similar in both groups (Table 17).

#### *Sputum purulence and volume*

There was no significant difference in sputum purulence between the groups at baseline or after 3 months treatment. At treatment months 6, 9 and 12 significantly fewer patients in the gentamicin group had purulent sputum compared with the saline group. At follow up, there was no difference in sputum purulence between the groups (Table 17).

There was no significant difference between the groups in 24hr sputum volume at any timepoint (Table 17).

Table 17. Comparison of nebulised gentamicin and nebulised saline on sputum characteristics.

<b>Sputum Characteristic</b>	<b>Month 0</b>	<b>Month 3</b>	<b>Month 6</b>	<b>Month 9</b>	<b>Month 12</b>	<b>Month 15</b>
<b>Sputum myeloperoxidase (units/ml)</b>						
Gentamicin Group	51.6 (29.8-132.6)	21 (3.4-50.3)	18.2 (7.7-41.2)	29.7 (9.9-41.1)	13.8 (3.-34.4)	47.7 (9.2-254.7)
Saline Group	40.5 (17.1-104.4)	44.6 (13.6-114.5)	52.1 (22.1-110.8)	49.8 (22.6-94.2)	58.2 (27-101.8)	67.5 (24.1-94.9)
Between group comparison P value	0.2	0.09	0.03	0.09	0.000	0.9
<b>Sputum free elastase activity (units/mg)</b>						
Gentamicin Group	3.6 (0-17.6)	0 (0-0)	0 (0-2.9)	0 (0-7.6)	0 (0-1.8)	7.1 (0-56.0)
Saline Group	4.1 (0-19)	0 (0-20.4)	0 (0-29.1)	0.9 (0-19.4)	1.8 (0.17-16)	2.8 (0.9-18.2)
Between group comparison P value	0.7	0.04	0.2	0.2	0.007	0.5
<b>% purulent sputum</b>						
Gentamicin Group	66.7	42.8	8.7	9.1	8.7	65.2
Saline Group	40.7	40	50	40	38.5	44
Between group comparison P value	0.09	0.06	0.02	0.01	0.02	0.16

Spinal Characteristic	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15
Between group comparison P value	0.1	0.1	0.08	0.3	0.9	0.13

### *Lung function and exercise capacity*

There was no significant difference between the groups in FEV<sub>1</sub>, FVC or FEF<sub>25-75</sub> at any timepoint (Table 18 and Figure 19).

There was no significant difference in exercise capacity between the groups at baseline or throughout the study until month 12 when exercise capacity had improved significantly more in the gentamicin group compared with the saline group. At follow up however, there was no significant difference between the groups (Table 18 and Figure 20).

Table 18. Comparison of nebulised gentamicin and nebulised saline on lung function and exercise capacity.

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15
<b>FEV<sub>1</sub>(L) %predicted</b>						
Gentamicin Group Actual	1.8 (1.34- 2.31)	1.74 (1.39- 2.28)	1.66 (1.32- 2.03)	1.63 (1.28- 2.07)	1.60 (1.34- 2.08)	1.69 (1.30-2.0)
%Predicted	72.9 (60-81.2)	70.5 (56.7- 88.2)	65.5 (53.4- 88.2)	69.1 (53.3- 76.6)	70 (55-82)	68.1 (55.7- 77.4)
Saline Group Actual	1.61 (1.22- 2.35)	1.68 (1.28- 2.23)	1.66 (1.22- 2.10)	1.61 (1.24- 2.15)	1.62 (1.24- 2.10)	1.67 (1.29- 2.16)
%Predicted	63.4 (45.5- 80.4)	64.9 (52-80.3)	66.2 (45.9- 81.4)	67.3 (46.4- 76.8)	61.5 (52.6- 80.5)	64.3 (48.4- 78.1)
Between group % predicted comparison P value	0.4	0.6	0.7	0.6	0.6	0.72
<b>FVC(L) %predicted</b>						
Gentamicin Group Actual	2.89 (2.21- 3.41)	2.86 (2.16- 3.29)	2.79 (2.2-3.28)	2.74 (2.11- 3.33)	2.65 (2.18- 3.11)	2.69 (2.19- 3.17)
%Predicted	88.9 (82.7- 94.1)	89.3 (78.9- 95.1)	87.1 (77.5- 92.1)	87.8 (75.6- 93.9)	87 (75.1- 92.7)	85.9 (80.7- 94.1)
Saline Group Actual	2.93 (2.12- 3.35)	2.83 (2.14- 3.35)	2.87 (2.25- 3.30)	2.68 (2.11- 3.33)	2.68 (2.20- 3.22)	2.67 (2.21- 3.20)
%Predicted	85 (71.1- 99.4)	89.4 (67.7- 96.9)	87.2 (69.8- 99.5)	86.9 (71-94.2)	85.7 (58- 103.4)	83..8 (66.3- 96.3)
Between group % predicted comparison P value	0.9	0.9	0.7	0.5	0.6	0.5

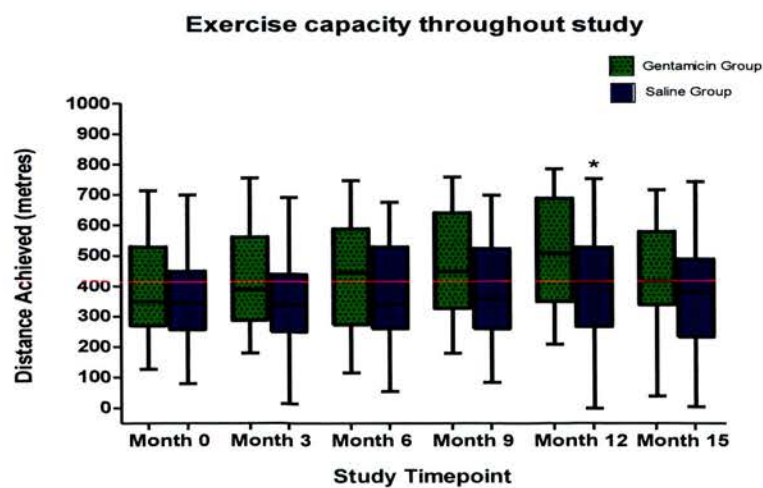
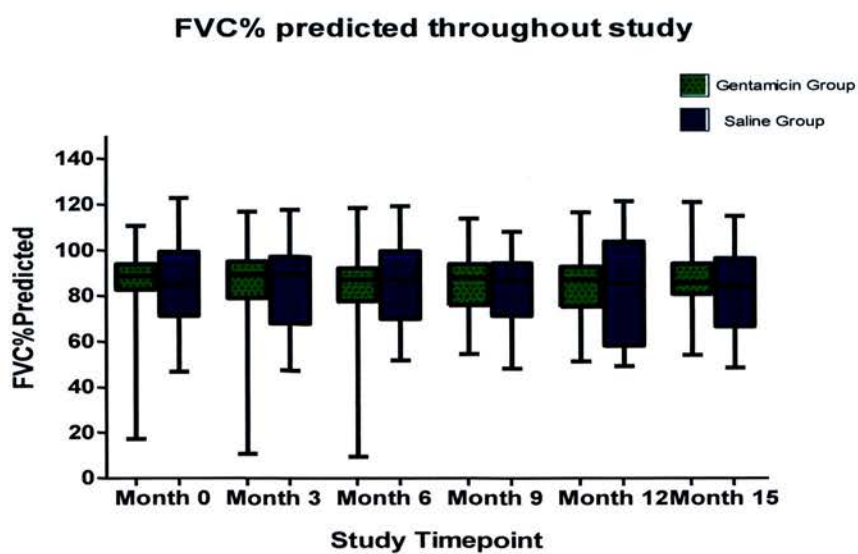
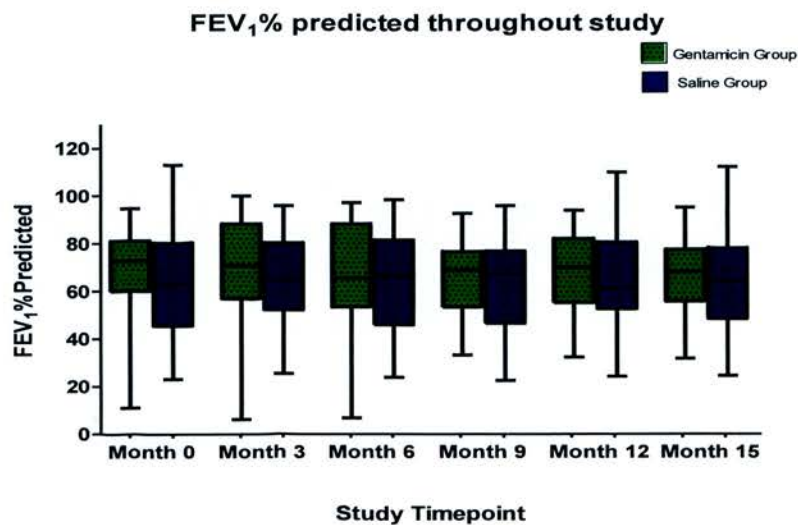
	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15
<b>FEF<sub>25-75</sub></b> (L.s <sup>-1</sup> )						
Gentamicin Group	1.06 (0.66- 1.62)	1.04 (0.65- 1.52)	0.77 (0.59- 1.44)	0.73 (0.59- 1.31)	0.9 (0.6-1.4)	0.93 (0.53- 1.48)
Saline Group	0.98 (0.49- 1.55)	0.85 (0.55- 1.52)	0.91 (0.52- 1.53)	0.74 (0.55- 1.49)	0.9 (0.58-1.6)	1.0 (0.52- 1.71)
Between group % predicted comparison P value	0.3	0.3	0.9	0.9	0.8	1.0
<b>FEV<sub>1</sub>/FVC</b> <b>%predicted</b>						
Gentamicin Group Actual(L)	0.67 (0.61- 0.78)	0.7 (0.55- 0.77)	0.63 (0.54- 0.68)	0.63 (0.54- 0.68)	0.65 (0.57- 0.75)	0.62 (0.55- 0.75)
%Predicted	84.5 (73.3- 91.0)	83.1 (68.3- 89.7)	79.7 (68.7- 95.3)	76.3 (63.2- 80.7)	78.3 (73.2- 88.3)	75.7 (69.3- 89.2)
Saline Group Actual(L)	0.65 (0.5-0.74)	0.62 (0.51- 0.73)	0.62 (0.5-0.7)	0.62 (0.5-0.72)	0.63 (0.51- 0.74)	0.64 (0.46- 0.73)
% Predicted	75.7 (58.6- 90.6)	76.9 (63.4- 86.2)	74.4 (63.4- 85.5)	73.5 (58.3- 88.6)	75.3 (62.7- 93.2)	78 (57.8- 88.4)
Between group % predicted comparison P value	0.9	0.9	1.0	0.8	0.4	1.0



	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15
<b>ISWT Distance (m)</b>						
Gentamicin Group	350 (270-530)	390 (287.5-562.5)	445 (272.5-590)	450 (372.5-642.5)	510* (350-690)	420 (340-580)
Saline Group	345 (257.5-450)	340 (250-440)	340 (260-530)	360 (260-525)	415 (267.5-530)	385 (232.5-490)
Between group comparison P value	0.4	0.06	0.2	0.08	0.03	0.3

The effect of nebulised gentamicin and nebulised saline on spirometry and exercise capacity is shown in Figure 20.

The effect on FEV1% predicted, FVC% predicted and exercise capacity throughout the study period in each group is shown. The horizontal lines represent the median and interquartile ranges and the whiskers represent the 5-95th percentile.



\*P=0.03

### *Health related quality of life*

At each 3 monthly interval during treatment, significantly more patients in the gentamicin group achieved a clinically significant improvement in both LCQ score and SGRQ score compared with patients in the saline group (Table 20). This effect was only sustained at follow-up with the SGRQ score. There was no significant difference seen at follow-up between the two groups with the LCQ score,  $P=0.5$ .

Table 19. Comparison of nebulised gentamicin and nebulised saline on LCQ and SGRQ.

	Month 3	Month 6	Month 9	Month 12	Month 15
<b>%LCQ<math>\geq</math>1.3unit improvement</b>					
Gentamicin Group	54.5*	68.1*	65*	81.4*	48
Saline Group	20	23.8	20	20	20
<b>%SGRQ <math>\geq</math>4unit improvement</b>					
Gentamicin Group	78.2**	81.8**	71.4**	87.5**	60.8**
Saline Group	31.8	34.7	23.8	19.2	19

The percentage of patients with a clinically significant improvement in LCQ and SGRQ score compared with their baseline score at entry to the study for each treatment timepoint and at follow-up is shown. At each timepoint, the difference between the groups was compared. \*P<0.01 \*\*P<0.004.

### *Systemic inflammation*

There was no significant difference in CRP, ESR or total leukocyte count between the groups at baseline. At months 3 and 9, CRP was significantly lower in the gentamicin group and at month 3 total leukocyte count was also lower in the gentamicin group. No other significant differences were seen in CRP and total leukocyte count at any timepoint during the study (Table 20). There was no significant difference between the groups in ESR at any timepoint.

Table 20. Comparison of nebulised gentamicin and nebulised saline on markers of systemic inflammation.

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15
<b>C-reactive Protein (mg/L)</b> (normal range <7mg.L)						
Gentamicin Group	7 (3-12)	2.5 (1-6)	3.5 (1-10.2)	3 (1.5-8)	4 (1.5-8.5)	7 (3-13)
Saline Group	7 (4-18)	6.5 (3.2-9.7)	5 (3-8)	8 (4-14.5)	7 (4-12)	7 (4-9)
Between group comparison P value	0.3	0.006	0.14	0.02	0.09	0.7
<b>Erythrocyte Sedimentation Rate (mm/hr)</b>						
Gentamicin Group	17 (11-29)	10 (7-18.7)	1 3(6-22.2)	14 (9-22)	17 (7.5-21)	17 (10-38)
Saline Group	16 (9-25.5)	10 (6-21.7)	12 (5.5-23)	12.5 (6.5-22.5)	14 (6.5-25.5)	11 (8-19)
Between group comparison P value	0.4	1.0	0.8	0.6	0.7	0.1
<b>Total leukocyte count (x10<sup>9</sup>/L)</b> (normal range 4-11x10 <sup>9</sup> /L)						
Gentamicin Group	7.3 (5.9-9.2)	5.8 (5-7.2)	6.6 (5.3-7.7)	6.5 (4.8-8.5)	7.1 (4.9-8.2)	6.4 (4.8-10.1)
Saline Group	7.7 (6.9-8.9)	7.2 (5.9-8.2)	6.8 (6.1-8.4)	7.7 (6.2-9.1)	7.1 (6.1-9.1)	7.5 (6.5-8.7)

Between group comparison P value	0.4	0.03	0.2	0.07	0.1	0.4
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### *Exacerbations*

There were fewer exacerbations during the 12 months' treatment in the nebulised gentamicin group compared with the saline group [0(0-1)exacerbations and 1.5(1-2)exacerbations respectively,  $P<0.0001$ ].

33.3% of patients in the gentamicin group had an exacerbation during the 12months' treatment compared with 80% in the saline group ( $P=0.0005$ ). The median time to first exacerbation during the 12months' treatment was 120(87-161.5) days in the gentamicin group compared with 61.5(20.7-122.7) days in the saline group,  $P=0.02$ .

There was no significant difference in the number of exacerbations that occurred during the 3 month treatment-free follow up period with 0(0-1) exacerbations occurring in the gentamicin group and 0(0-1) exacerbations occurring in the saline group.

### *Gentamicin toxicity*

Serum gentamicin levels at the end of treatment were 0.13(0-0.37)mg/ml. Only one patient developed a serum gentamicin level  $>1$ mg/ml during the study. This occurred at treatment month 9 (level 1.25mg/ml) and following reduction of study treatment to once daily all subsequent levels (at weekly intervals for 3 weeks) were  $<1$ mg/ml for the subject concerned.

### *Adverse Effects*

In the total cohort originally allocated to nebulised gentamicin, 21.9% (7 of 32 patients) reported bronchospasm and received adjunctive nebulised  $\beta_2$  agonist treatment. Despite this, two patients required withdrawal from the study (one at month 3 and one at month



6) as they remained unable to tolerate the treatment. The remaining 5 patients successfully completed the study and were included in the final analysis.

In the total cohort originally allocated to nebulised saline, 6% (two of 33 patients) reported bronchospasm and received adjunctive nebulised  $\beta_2$  agonist treatment. Despite this, both required withdrawal from the study (both at month 3) as they remained unable to tolerate the treatment. 16.7% (five patients) in the saline group who completed the study reported a salty taste with nebulisation.

No nephrotoxicity was detected and no ototoxicity reported. 11.1% of patients in the gentamicin group (three patients) reported an unpleasant taste following nebulisation.

#### *Compliance*

One patient in the gentamicin group had compliance issues. On counting of total drug doses returned, by the end of the 12 months' treatment this study patient had taken only 67.9% of the total doses prescribed throughout the study. This patient was included in the final analysis. There were no issues with compliance in the saline group.

#### *Changes to routine respiratory medication*

No changes were made to any patient's routine respiratory therapy during the study.

## **5.4 Discussion**

This randomised controlled trial has proven efficacy for nebulised gentamicin in non-cystic fibrosis bronchiectasis. Regular, long-term nebulised gentamicin significantly reduces sputum bacterial density and airways inflammation, with less sputum purulence. Patients had greater exercise capacity, fewer exacerbations and an improved health related quality of life.

The pathophysiological basis of bacterial infection and airways inflammation in bronchiectasis was originally proposed by Cole et al approximately two decades ago and offers a clear target for therapeutic intervention (Cole, 1986). Nebulising antibiotic therapy allows direct treatment to the damaged airways and with localised treatment, systemic side effects are limited (Palmer et al., 1998). Gentamicin was selected as it is broad spectrum for the pathogens seen in non-cystic fibrosis bronchiectasis, inexpensive (current cost for a 12 month 80mg twice daily course totals £1124.20) and has previously been shown to achieve good sputum concentrations (Crowther Labiris et al., 1999, Twiss et al., 2005a).

There have been no previous long term studies of nebulised gentamicin in bronchiectasis and the optimum dose and regime is unknown. There has been one study to date exploring the dosage of nebulised gentamicin in non-cystic fibrosis bronchiectasis in adults (Crowther Labiris et al., 1999). Crowther Labiris et al found that a single dose of 160mg of nebulised gentamicin, achieved good sputum concentrations  $[97.2(0.3-194.2)\mu\text{g/g sputum}]$  two hours following nebulisation (Crowther Labiris et al., 1999). There were however, reports of an unpleasant taste and mouth irritation following nebulisation. Although there have been no previous long

term studies, anecdotal opinions of nebulised gentamicin in bronchiectasis have preferred 80mg twice daily dosing (Currie, 1997). In the 2 years prior to the commencement of this study, a small pilot study (n=5) was conducted within the bronchiectasis clinic at the Royal Infirmary of Edinburgh, using 80mg of nebulised gentamicin twice daily and found it to be well tolerated with no reported bronchoconstriction and no detectable serum concentrations. For this study, a regime of 80mg twice daily was selected as a dose likely to achieve adequate sputum concentrations with minimal adverse effects and systemic toxicity, particularly for long term administration.

The majority of patients with bronchiectasis are chronically infected with pathogenic bacteria in their airways even when apparently clinically stable. Angrill et al, in their study of 77 patients, found 64% to be colonised, most frequently with *Haemophilus influenzae* (55%) followed by *Pseudomonas aeruginosa* (26%) (Angrill et al., 2002). King et al conducted a prospective 5 year follow up study of 89 patients with bronchiectasis, of whom 79% had pathogens in their sputum when clinically stable (*Haemophilus influenzae* in 47%, *Pseudomonas aeruginosa* in 26%, with *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus* responsible for infection the remaining 27%) and found that the infecting pathogen varied little over the 5 year follow-up period (King et al., 2007). Previous randomised controlled trials of aerosolised antibiotics have included only patients colonised with *Pseudomonas aeruginosa*- our study was unique to include patients infected with a variety of pathogens and our baseline demographics support the previous studies in terms of

incidence of infecting pathogen (Orriols et al., 1999, Barker et al., 2000, Drobnic et al., 2005).

Angrill et al in a separate study, evaluated the relationship between bacterial infection and airways inflammation using bronchoalveolar lavage in both infected and non-infected patients with bronchiectasis as well as control subjects (Angrill et al., 2001). Infected patients had an exaggerated inflammatory response (greater number of neutrophils, higher concentrations of elastase, myeloperoxidase and other inflammatory markers) compared with non-infected patients. The diagnostic cut-off value used to define bacterial infection in bronchial washings in the study was  $10^3$ cfu/ml, and with a one log unit increase, at  $10^4$ cfu/ml, there was a significant increase in airway inflammatory markers. Hill et al found similar results using sputum in patients with chronic bronchitis and bronchiectasis, specifically demonstrating a significant increase in markers of airways inflammation with sputum bacterial loads of  $\geq 10^6$ cfu/ml (Hill et al., 2000). Prior to entering the current study, all patients had significant bacterial density in their sputum with a median of  $8.02 \log_{10}$ cfu/ml in the gentamicin group and  $7.88 \log_{10}$ cfu/ml in the saline group with high levels of MPO and free elastase activity when clinically stable. The primary endpoint was a greater than or equal to one log unit reduction in sputum bacterial load, regarded as the minimum important reduction necessary to have a significant impact on airways inflammation. A significant reduction in bacterial density was seen after 3 months of nebulised gentamicin, an effect sustained throughout the 12 months' of treatment. Similar to Barker et al's study (Barker et al., 2000), in the gentamicin group pathogen eradication was achieved in 30.8% of patients colonised with *Pseudomonas aeruginosa* and in those infected by other pathogens,

eradication was achieved in 92.8%. In those not achieving complete eradication of pathogens, there was still a significant reduction in bacterial density seen. Furthermore, parallel reductions in sputum MPO was seen, although not significantly so until treatment month 6. Free elastase activity also significantly reduced after 3 months' treatment. These treatment effects further corroborate Cole's hypothesis of infection and inflammation in bronchiectasis (Cole, 1986). In the saline group, there was no change in bacterial density or infecting pathogen and airways inflammation seen throughout the study.

Emerging strains of antibiotic resistant bacteria is an increasing problem in lung infection. In bronchiectasis, the potential virulence of the pathogens typically responsible for chronic bronchial infection combined with the repeated courses of antibiotics required for exacerbations makes the potential development of antimicrobial resistance an almost expected phenomenon, but one that poses a real threat to a chronically unwell population with significant disease burden. To date, there is little data available regarding the impact of long term inhaled antibiotics on susceptibility patterns in non-cystic fibrosis bronchiectasis. Three studies of nebulised tobramycin in patients chronically infected with *Pseudomonas aeruginosa* have included observation of emergence of tobramycin resistant *Pseudomonas* strains (Couch, 2001, Scheinberg and Shore, 2005, Drobic et al., 2005). Couch et al found resistance in 11% of patients following 4 weeks' treatment, although 3% of those treated with placebo also developed resistance (Couch, 2001). Scheinberg et al noted that after 12 weeks' nebulised tobramycin, 7% developed a resistant *Pseudomonas aeruginosa* (Scheinberg and Shore, 2005). Drobic et al in their inhaled tobramycin study found resistance developed

during treatment in 10% of patients, but they also observed resistance developing during the placebo period in 10%; reassuringly two months following completion of the study, all patients had tobramycin susceptible strains of *Pseudomonas aeruginosa* (Drobnic et al., 2005). In this current study, all patients chronically infected with *Pseudomonas aeruginosa* or Gram negative enteric organisms were fully susceptible to gentamicin prior to entry to the study and there was no emergence of resistant strains. No patient had a gentamicin resistant *Pseudomonas* strain or Gram negative enteric bacterial isolate at the end of the twelve months or indeed at follow up, three months following completion of treatment. However, a recent study by Gillham et al found variation in morphotype and antibiotic susceptibility in colonies of *Pseudomonas aeruginosa* isolated from non-cystic fibrosis bronchiectasis sputum indicating that further studies are needed to explore the relevance and clinical implications of in vitro susceptibilities in these complex, chronically infected patients with recurrent exacerbations (Gillham et al., 2009). A limitation of this study is that susceptibility testing was not done more frequently throughout the 12 months' treatment, as it is possible that changes in zone diameter not reaching defined resistance diameters may have occurred. Furthermore, only *Pseudomonas* strains and Gram negative bacterial isolates were tested for gentamicin susceptibility; gentamicin susceptibility was not routinely tested for in all other potentially pathogenic microorganisms isolated.

Purulent sputum in stable bronchiectasis is associated with bacterial infection, varicose or cystic airway dilatation and an  $FEV_1 < 80\%$  predicted (Murray et al., 2009b). Increased sputum purulence is also associated with increased neutrophilic airways inflammation in patients with chronic bronchitis and bronchiectasis (Stockley et al.,

2001). In this study, two-thirds of patients in the gentamicin group had purulent sputum at baseline but this had significantly reduced to 8.7% after 6 months' treatment and was sustained for the remainder of the gentamicin treatment. No such similar effect was seen in the saline group where 40% of patients had purulent sputum at baseline and 38.5% after 12 months' treatment. No significant change was however observed in the volume of sputum expectorated over 24hrs between the groups throughout the study. It is questionable how reliable this endpoint is, as factors such as patient compliance, swallowed secretions and collection of saliva may affect it.

The correlation between airways inflammation and systemic inflammation has previously been shown to be poor (Angrill et al., 2001). In this study, despite good improvement in bacterial clearance, there was no significant change in systemic inflammation between the groups at the end of 12 months' treatment.

Exercise capacity has been used as a marker of treatment response in previous interventional studies with regular chest physiotherapy and treatment of exacerbations having significant improvement on distance achieved in the incremental shuttle walk test (Murray et al., 2009a, Murray et al., 2009c). Lin et al in their randomised controlled trial of nebulised gentamicin over 3 days found a significant improvement in patients' 6 minute walk test (Lin et al., 1997). In this study, after 12 months' treatment, patients receiving nebulised gentamicin had significantly improved their exercise capacity compared with those receiving nebulised saline. The distance achieved improved by a median of 160m, compared with baseline. However, despite this improvement in exercise capacity, no similar effects were seen in FEV<sub>1</sub>, FVC or mid-expiratory flows. The value of spirometric makers as a measure of treatment effect in non-cystic fibrosis



bronchiectasis is questionable. Orriols et al in their randomised controlled trial of nebulised ceftazidime and tobramycin in 15 patients also observed no change in FEV<sub>1</sub> or FVC (Orriols et al., 1999). Previous trials of other long term interventional studies including inhaled corticosteroid therapy and regular chest physiotherapy have also not observed any changes in FEV<sub>1</sub> (Tsang et al., 2005a, Murray et al., 2009a).

Infective exacerbations are a significant cause of morbidity and often necessitate utilisation of both primary and secondary health care resources (Kelly et al., 2003). Recurrent exacerbations are associated with a poor health related quality of life and add further insult to the persistently damaged, inflamed airways. Reducing exacerbations is a major goal of management in this chronic condition with previous long term antibiotic studies evaluating the impact of treatment on exacerbations: Orriols et al found a reduced number of hospital admissions and inpatient days with 12 months nebulised ceftazidime and tobramycin; Drobic et al found a reduced number of exacerbations requiring hospital admission and for those admitted a shorter length of stay in their 6 month randomised controlled trial of nebulised tobramycin (Orriols et al., 1999, Drobic et al., 2005). In this study, patients receiving nebulised gentamicin had fewer exacerbations during treatment and took twice as long to their first exacerbation during the treatment phase than patients in the saline group.

With significant reductions in sputum bacterial density and inflammation as well as improved exercise capacity and fewer exacerbations, significantly more patients in the gentamicin group had a clinically significant improvement in health related quality of life as assessed by both the LCQ and the SGRQ, arguably the most important goal of management in chronic disease. However, within the saline group a small percentage of



patients also had an improvement in HRQL. This would suggest a small physiotherapy effect or a potential placebo effect as a consequence of either a new treatment, the impact of the frequent study reviews or immediate access to tertiary care support if they felt unwell.

Nebulised gentamicin was a reasonably well tolerated therapy, with few adverse effects. Despite no significant bronchospasm with the initial gentamicin challenge, seven of the original 32 patients subsequently developed bronchospasm. These patients received adjunctive nebulised beta-2-agonist and five were able to continue with the nebulised gentamicin and complete the study. However, in Barker et al's study of 4 weeks of nebulised tobramycin there was an overall increased incidence of reported wheeze, cough, chest pain and dyspnoea with treatment- 70% of patients reported at least one respiratory symptom (Barker et al., 2000). No patient suffered other adverse effects commonly related to gentamicin, particularly there were no cases of nephrotoxicity or ototoxicity. Only one patient developed a significantly detectable serum level of gentamicin at month 9, requiring a reduction in treatment dose to 80mg once daily. These findings would suggest that with close monitoring, nebulised gentamicin is a safe treatment.

Despite significant benefits with nebulised gentamicin throughout 12 months' treatment, it was disappointing that none of the treatment effects were sustained in the 3 month treatment free follow up period, with sputum characteristics returning to values similar to baseline. Only one randomised controlled trial of nebulised antibiotics has previously included a follow up period during which time eradication of *Pseudomonas aeruginosa* from the sputum was sustained, but this was only over 2 weeks follow-up

period and it is not known whether this effect would be sustained over a longer follow-up period (Barker et al., 2000). Although our findings during the follow-up period are disappointing, they provide clear guidance for clinicians that for treatment with nebulised gentamicin to be effective, it must be continuous. Further studies are however, needed to decide on the optimum regime.

There is a paucity of evidence for effective long term treatments in non-cystic fibrosis bronchiectasis. This randomised controlled trial has shown that regular nebulised gentamicin can significantly reduce sputum bacterial infection, reduce airways inflammation, improve sputum purulence, improve exercise capacity, reduce exacerbations and improve health related quality of life in chronically infected patients with bronchiectasis, irrespective of infecting pathogen.

The final study sought to explore whether there are any key parameter- either clinical or laboratory markers- that can be used to assess response to long term treatment strategies in the management of bronchiectasis.

**CHAPTER SIX:**  
**ASSESSING RESPONSE TO TREATMENT IN BRONCHIECTASIS**

## **6.1 Introduction**

Bronchiectasis is a chronic, debilitating illness responsible for significant patient morbidity and substantial socioeconomic cost (Martinez-Garcia et al., 2005, Weycker D, 2005, Kelly et al., 2003). Efficacious treatments are necessary but it is poorly understood how best to monitor response. Few clinical or laboratory markers have been specifically validated for the disease with previous interventional and observational studies typically relying upon indices proven for other chronic respiratory conditions, particularly chronic obstructive pulmonary disease (COPD) and cystic fibrosis. More recently the reliability or relevance of such methods has been questioned (Athanasio et al., 2010).

There are currently two clinical outcome measures validated for use in bronchiectasis. Both are health related quality of life questionnaires, the St George's Respiratory Questionnaire (SGRQ) (Jones et al., 1992, Wilson et al., 1997a) and the Leicester Cough Questionnaire (LCQ) (Birring et al., 2003, Murray et al., 2009d). Improving health related quality of life is arguably the most important goal of management in non-fatal yet incurable chronic conditions, however objective assessment of health related quality of life with such questionnaires may be time consuming and cumbersome for both the patient to complete and the clinician to analyse, thus limiting its use in daily practise. Other pertinent, non-invasive, inexpensive and reliable outcome measures are urgently needed (Chang and Bilton, 2008).

The aim was to identify parameters that may be useful in assessing response to chronic interventions in stable disease. A clinically significant improvement in the total St George's Respiratory Questionnaire was considered an effective response to treatment

and used to define a successful treatment outcome against which other currently non-validated measures were assessed including: forced expiratory volume in one second (FEV<sub>1</sub>), forced vital capacity (FVC), mid-expiratory flows (FEF<sub>25-75</sub>), incremental shuttle walk test (ISWT), sputum bacteriology, sputum colour, total white cell count (WCC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and exacerbation frequency.

## **6.2 Methods**

An analysis of outcome measures used to assess response to chronic intervention in stable disease (treatment with regular chest physiotherapy compared with no regular chest physiotherapy over three months and treatment with regular nebulised gentamicin compared with nebulised 0.9% saline over 12 months) was conducted.

Patients involved in the preceding studies were included in the analysis and classified as “responders” or “non-responders”.

*Responders:* Patients achieving an improvement of  $\geq 4$  units in their SGRQ total score at the end of the study when compared with their baseline SGRQ total score at the start of the study. A change of  $\geq 4$  units is the minimal clinically important difference for change (Wilson et al., 1997a).

*Non responders:* patients who failed to achieve an improvement of  $\geq 4$  units in their SGRQ total score at the end of the study when compared with their baseline SGRQ total score at the start of the study.

The following outcome measures were included in the analysis of response to treatment: FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, ISWT, quantitative sputum bacterial load, qualitative sputum bacterial clearance, sputum colour, WCC, ESR, CRP and exacerbation frequency. Cut-offs were determined using the results from the previous study of infective exacerbations described in Chapter Two.

All outcome measures were assessed as described in the General Methods section.

### *Statistics*

All statistics were performed using SPSS for Windows, Version 17 (SPSS inc, Illinois).

For comparisons between responders and non-responders, a Mann-Whitney U test was performed. A multivariate forward logistic regression analysis was performed to assess the predictors of response to long term treatment strategies in stable bronchiectasis. A P value of  $<0.05$  was used for entry into the model. Multicollinearity was assessed using the variance inflation factor (VIF) with a  $VIF < 2.5$  regarded as excluding any significant interaction. The dependant variable was an improvement in SGRQ of  $\geq 4$  units. The independent variables were:  $>12\%$  and  $>200\text{ml}$  increase in  $FEV_1$ ;  $>300\text{ml}$  increase in FVC;  $>20\%$  increase in  $FEF_{25-75}$ ;  $\geq 50\text{m}$  improvement in the distance achieved in ISWT;  $\geq 1$  log cfu.ml reduction in bacterial load;  $\geq 50\%$  reduction in WCC;  $\geq 50\%$  reduction in ESR;  $\geq 75\%$  reduction in CRP;  $\geq 1$  point improvement in sputum colour chart measurement; complete eradication of bacteria from the sputum;  $\leq 2$  exacerbations during intervention. A second multivariate analysis was performed using the same model but excluding complete eradication of bacteria from the sputum as an independent variable to identify other independent predictive factors accounting for patients with chronically infected sputum who may therefore be less likely to achieve complete sputum bacterial eradication but may otherwise respond to treatment. A 2-tailed P value of  $<0.05$  was considered statistically significant.

### **6.3**    **Results**

#### *Baseline comparison between responders and non-responders*

There were 39 responders and 52 non-responders. There was no significant difference in age, sex, % predicted FEV<sub>1</sub>, % predicted FVC, % predicted FEV<sub>1</sub>/FVC ratio or radiological extent of disease between responders and non-responders when clinically stable prior to the intervention (Table 21).



Table 21. Baseline characteristics of responders and non-responders to treatment.

<b>Characteristic</b>	<b>Responders n=39 n(%) Median(IQR)</b>	<b>Non-responders n=52 n(%) Median(IQR)</b>	<b>P</b>
<b>Male sex</b>	21(53.8)	28(53.8)	0.8
<b>Age (years)</b>	67(58-72)	71(64.2-75.0)	0.08
<b>Ex-smoker</b>	14(35.9)	20(38.5)	1.0
<b>% Predicted FEV<sub>1</sub></b>	72.2(57.2-81.3)	69.0(47.9-88.5)	0.6
<b>% Predicted FVC</b>	86.3(78.7-98.6)	84.1(70.0-98.3)	0.6
<b>FEV<sub>1</sub>/FVC % Predicted</b>	63.5(55.2-81.2)	68(56.2-78.3)	0.9
<b>Number of lobes affected on CT</b>	4(3-6)	4(3-5)	0.7
<b>Presence of cystic dilatation</b>	23(58.9)	23(44.2)	0.1

The characteristics of responders and non-responders when clinically stable prior to entry into respective intervention studies are shown.

*Effect of intervention on variables in responders and non-responders*

There was a significant difference between responders and non-responders to treatment in FVC, ISWT, sputum colour, sputum bacterial eradication and sputum bacterial load (Table 22).

Table 22. Effect of treatment on outcome variables for responders and non-responders to treatment.

<b>Outcome Variable</b>	<b>Responders n= 39 n(%) or Median(IQR)</b>	<b>Non-responders n=52 n(%) or Median(IQR)</b>	<b>P Value</b>
<b>Change in FEV1 (L)</b>	-0.08(-0.2-0.01)	-0.02(-0.1-0.09)	0.07
<b>Change in FVC (L)</b>	-0.11(-0.33-0.07)	0.06(-0.18-0.21)	0.02
<b>Change in FEF<sub>25-75</sub>(L)</b>	-0.14(-0.43-0.02)	-0.01(-0.15-0.14)	0.06
<b>Change in incremental shuttle walk test (metres)</b>	70(20-130)	10.0(-10-40)	<0.0001
<b>1 point improvement in sputum colour</b>	16(41)	7(13.4)	0.004
<b>Change in bacterial load (log<sub>10</sub>cfu/ml)</b>	-2.24(-6.6- -0.05)	-0.55(-0.88-0.18)	0.008
<b>Bacteria eradicated from sputum</b>	15(38.4)	2(3.8)	<0.0001
<b>Change in total white cell count (x10<sup>9</sup>/L)</b>	0.5(-0.87-2.07)	0.0(-0.8-1.2)	0.3
<b>Change in erythrocyte sedimentation rate (mm/hr)</b>	2.0(-7.0-7.7)	0.0(-6.5-7.5)	0.5
<b>Change in C-reactive protein (mg/L)</b>	1.0(-1.0-6.0)	0.0(-2.7-3.7)	0.09
<b>Change in exacerbation frequency</b>	0(0-1)	0(0-1)	0.07

*Independent factors predictive of a successful response to treatment*

All factors had a VIF of  $<2.0$ . Independent factors were an improvement in the distance achieved in the ISWT by  $\geq 50\text{m}$  and eradication of bacteria from the sputum (Table 23).

Table 23. Factors predictive of a response to treatment.

<b>Factor</b>	<b>Odds ratio</b> (Upper and lower 95% Confidence Interval)	<b>P Value</b>
<b>≥50m improvement in ISWT</b>	5.6(1.3-24.2)	0.02
<b>Eradication of bacteria from sputum</b>	7.7(1.2-49.2)	0.03

Multivariate logistic regression analysis of factors predictive of a successful response to chronic treatment in bronchiectasis.

Independent factors predicting a response to treatment with sputum bacterial eradication excluded from the model were an improvement in the distance achieved in the ISWT by  $\geq 50\text{m}$  and improvement in sputum colour by  $\geq$  one point on the sputum colour chart (Table 24).

Table 24. Factors predictive of a response to treatment excluding sputum bacterial eradication.

<b>Factor</b>	<b>Odds ratio (Upper and lower 95% Confidence Interval)</b>	<b>P Value</b>
<b>≥50m improvement in ISWT</b>	5.76(1.9-17.3)	0.006
<b>≥1 point improvement in sputum colour</b>	3.5(1.03-11.8)	0.04

Multivariate logistic regression analysis of factors predictive of a successful response to chronic treatment in bronchiectasis excluding complete sputum bacterial eradication.

## **6.4 Discussion**

Measures that may be important in the assessment of a successful response to long-term treatments in bronchiectasis include an improvement in the incremental shuttle walk test, eradication of bacteria from the sputum, a reduction in sputum bacterial density and an improvement in sputum purulence. Independent outcome measures that may predict a successful response to chronic treatment strategies (defined as  $\geq 4$  unit improvement in SGRQ total score) include an improvement in the incremental shuttle walk test of  $\geq 50$ m and eradication of bacteria from sputum.

Patients who had a successful response to treatment achieved a significantly greater distance in the ISWT than those who failed to respond to treatment. Exercise capacity is highly relevant in bronchiectasis as it has long been recognised to promote airway clearance (Macmahon, 1915) and more recently, a poor score in the activities section of the SGRQ has been proven to be a predictor of mortality (Loebinger et al., 2009b). Previous studies recognising exercise capacity as an important outcome include Lin's study of twice daily inhaled gentamicin improving the distance achieved in the 6-minute walk test and Newall et al in their study of exercise training and inspiratory muscle training in patients with bronchiectasis used the ISWT as an outcome measure, recognising the importance of exercise tolerance and health related quality of life (Newall et al., 2005). In both models of the multivariate analysis, a minimum improvement of 50m was found to be an independent predictor of a successful treatment outcome. An improvement of 50m was selected based on the utility of the ISWT in acute exacerbations (Murray et al., 2009c). The ISWT is an inexpensive, easily conducted assessment with real pertinence to bronchiectasis in terms of both health related quality of life and mortality. Further external validation studies are needed.



Chronic airways infection is associated with increased airways inflammation, a poorer health related quality of life, more frequent exacerbations and with chronic *Pseudomonas* infection, a more rapid decline in lung function (Martinez-Garcia et al., 2007, Angrill et al., 2001, Courtney et al., 2008). Limiting airways infection is a major goal of long term management in bronchiectasis. Sputum bacteriology has been used as an outcome measure in previous interventional studies for both acute management strategies and in the evaluation of long term therapies (Stockley et al., 1984a, Tsang et al., 1999a). This study included two different measures of sputum bacteriology as potential predictors of treatment response. Qualitative bacteriology reports the presence or absence of pathogens in sputum and is the most frequent technique utilised by laboratories to measure airways infection. Quantitative bacteriology provides an accurate assessment of bacterial density in the infected airways and in patients with chronic infection unlikely to achieve complete pathogen eradication, quantitative bacteriology measuring any impact or reduction in density may be a more useful measure. Univariate analysis identified both measures as significant discriminators of successful treatment response. On multivariate analysis, quantitative bacteriology measuring complete eradication of bacterial pathogens from sputum emerged as the more powerful measure as an independent marker of a successful treatment response. With complete pathogen eradication, patients achieved an improved health related quality of life and a successful treatment response.

Despite not achieving complete sputum pathogen eradication, particularly in patients with chronic airways infection, a successful treatment response may still be achieved. The patients included in the interventional studies analysed were all required to have

evidence of chronic airways infection for eligibility for study entry. A second, separate analysis of the model was therefore undertaken excluding complete pathogen eradication as a potential independent predictor to assess for other measures that may predict a successful treatment response. This identified a one point improvement in sputum purulence in addition to an improvement in exercise capacity ( $\geq 50$ m in the ISWT) as independent predictors of treatment response.

Sputum purulence reflects airways inflammation and is positively associated with bacterial infection (Stockley et al., 2001, Murray et al., 2009b). Changes in sputum purulence have been utilised as measures of treatment response since the earliest observational reports of management of bronchiectasis and in the earliest randomised controlled treatment trials (1957, Cherniack et al., 1959, Hill et al., 1988, Currie et al., 1990). In the multivariate analysis that excluded complete pathogen eradication as a potential predictor of treatment response, an improvement in sputum purulence emerged as an independent predictor of a successful treatment response. With clearer sputum, there is less airways infection and inflammation and an improved health related quality of life. Sputum purulence is a non-invasive, inexpensive and pertinent marker that can be reliably measured and reported (Murray et al., 2009b).

Lung function- particularly FEV<sub>1</sub> and FVC- are frequently and reliably used as markers of treatment efficacy in the management of other chronic respiratory diseases such as cystic fibrosis and asthma. To date, studies in bronchiectasis have failed to provide convincing support for lung function as a useful marker of treatment response. Orriols et al in their randomised controlled trial of nebulised ceftazidime and tobramycin in 15 patients observed no change in FEV<sub>1</sub> or FVC (Orriols et al., 1999). Tsang et al observed

no change with 12 months' inhaled corticosteroid treatment (Tsang et al., 2005a). However, some studies of acute exacerbations have noticed significant improvements in FEV<sub>1</sub> and FVC with an earlier open label study found that following 2 weeks of treatment with oral amoxicillin there were small improvements in FEV<sub>1</sub> (mean improvement 8mLs), FVC (mean improvement 180mLs), vital capacity (mean improvement 170mLs), functional residual capacity (mean improvement 160mLs) and total lung capacity (mean improvement 230mLs) although these changes were not felt to be clinically useful (Hill and Stockley, 1986). The clinical relevance of any change in FEV<sub>1</sub> and FVC was explored in the multivariate model using an improvement of 200mls and  $\geq 12\%$  increase from baseline in FEV<sub>1</sub> and an improvement of >300mls in FVC based on the current American Thoracic Society/European Respiratory Society guidelines for interpretation of lung function (Pellegrino et al., 2005). These changes were not found to be independent predictors of a successful treatment response and the utility of FEV<sub>1</sub> and FVC in measuring treatment response remains unproven with little evidence to currently support any further investigation of their role.

There was no significant difference in WCC, ESR or CRP observed between responders and non-responders. Cut-off levels for a clinically relevant change in these markers were based on the role of these markers in acute exacerbations (Murray et al., 2009c), however none were found to be useful predictors of a successful response to chronic treatment strategies. These markers are more likely to have a role in assessing interventions in the management of acute exacerbations of bronchiectasis which are typically accompanied by a systemic inflammatory response (Murray et al., 2009c).

The analysis also included exacerbation frequency as a measure of treatment response. Exacerbations are a stimulus to the vicious cycle of infection and inflammation in the already damaged bronchiectatic airways and adversely impact on health related quality of life- reducing their frequency is a major goal of management. On multivariate analysis it was not an independent predictor of treatment response. This should however be interpreted with caution as larger, multicentre trials would be necessary to address this as an outcome measure.

Bronchiectasis is responsible for significant morbidity and frequent utilisation of health care resources. Over the past decade it has become increasingly recognised that effective, evidence-based treatment strategies are necessary. To effectively assess a treatment strategy clear and validated endpoints are needed. Improvement in health related quality of life is a key endpoint for all interventions. This study has found that sputum microbial clearance and increasing exercise capacity as measured by an increase of  $\geq 50$ m in the ISWT as well as improved sputum purulence are independent predictors of a successful treatment response. Further external validation studies of these markers are needed.

**CHAPTER SEVEN:**  
**GENERAL DISCUSSION**

### **7.1 Augmentation of the mucociliary system**

In bronchiectasis the normal mucociliary clearance mechanism is impaired causing disruption of both the integrity and sterility of the bronchial tree. Early studies of inhaled labelled aerosols demonstrated abnormal and slow rates of clearance from the airways of patients with bronchiectasis (Lourenco et al., 1972, Svartengren et al., 1986, Isawa et al., 1990). Excessive, viscous and purulent secretions accumulate, causing an increased risk of invasion and chronic colonisation of the bronchial mucosa by potentially pathogenic microorganisms resulting in perpetual airways inflammation and constant stimulation of the vicious cycle (Cole, 1986). The rationale for chest physiotherapy is to loosen secretions and aid their movement through the bronchial tree as well as enhancing expectoration. With fewer secretions in the airways there may be less exposure of the bronchial mucosa to toxins and pathogens with less perpetuation of the vicious cycle of infection and inflammation, improved symptom control and improved disease parameters. Despite the plausibility of this rationale and the routine recommendation for its use, there is a paucity of evidence for the efficacy of physiotherapy in bronchiectasis.

The study in Chapter Three explored the impact of regular twice daily chest physiotherapy with no chest physiotherapy in clinically stable patients with bronchiectasis. The aim was to assess whether augmentation of the mucociliary clearance mechanism could achieve one of the major goals of management in bronchiectasis by improving patients' health related quality of life and to assess its impact on other disease parameters to help evaluate whether it is a treatment strategy that may interrupt the vicious cycle of infection and inflammation.

In this crossover study 20 patients who did not normally practise regular physiotherapy were treated for 3 months with twice daily chest physiotherapy using an oscillatory positive expiratory pressure device. Significant differences were observed with regular chest physiotherapy including an improvement in perceived cough severity measured with the LCQ score, an increase in 24 hour sputum volume, an improvement in exercise capacity and an improved health related quality of life as quantified by SGRQ score. There was no significant effect on sputum microbiology, FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, MIP, MEP or exacerbation frequency.

Regular chest physiotherapy achieves one of the main goals of management of bronchiectasis, an improvement in health related quality of life. Improvements were seen both in the impact of cough severity measured using the LCQ and in the effect of general respiratory symptoms on health related quality life using the SGRQ. The changes observed with regular chest physiotherapy were clinically significant with a median improvement in LCQ total score of 1.3units, and a median improvement of 7.8 units in the SGRQ total score. It is of particular relevance that within the SGRQ score, a significant improvement in the activities score was seen as this section of the SGRQ has been identified as an independent risk factor for mortality (Loebinger et al., 2009b).

The rationale that chest physiotherapy should aid mobilisation of secretions is proven clinically in this study with the increase in sputum volume expectorated over 24hours. A median increase of 2mls in 24hour sputum volume expectoration with regular chest physiotherapy was observed, implying better mobilisation of secretions. Although the current study described in Chapter Three did not intend to explore the mechanistic action of regular physiotherapy, the direct effect of physiotherapy on mucociliary

transportation in bronchiectasis has been investigated in a recent 4 week crossover study. 18 patients were exposed to a regime of a daily 30 minute physiotherapy session with a high frequency oscillatory positive expiratory pressure device (the Flutter VRP1) followed by a washout period then a regime of a daily 30 minute session with positive expiratory pressure physiotherapy. Sputum samples were collected on a weekly basis and analysed in a cough simulated machine for contact angle measurement and relative transport velocity. The combination of high frequency oscillation and positive expiratory pressure resulted in a greater displacement of expectorated sputum with a lower contact angle measurement, but no significant difference was seen in the relative transport velocity (Tambascio et al., 2011). This contrasts with an earlier evaluation of sputum expectorated following a single session of either the Flutter VPR1 or positive expiratory pressure or no physiotherapy which found no change in ciliary or cough transportability or contact angle measurements between all three groups (Valente, 2004). The difference in observations between the two studies suggests that regular, long term practice of daily physiotherapy may be important for treatment efficacy.

It is intriguing that one of the aims of physiotherapy is to increase secretion expectoration but that studies of other treatment strategies have typically always sought a reduction in sputum expectoration as an indication of improved symptoms and a measure of treatment efficacy. Longer studies are needed to establish whether physiotherapy initially clears the airways of secretions causing perhaps a transient increase in sputum volume followed by a reduced volume in the longer term.

The increased sputum clearance together with subjective better control of cough reflected by the improvement in the LCQ may be sufficient to contribute to the



improvement observed in exercise capacity but is not reflected in physiologic improvement of airways obstruction with the absence of any improvement in mid expiratory flows, FEV<sub>1</sub> or FVC.

Exercise capacity was assessed using the incremental shuttle walk test and a median improvement of 40m with regular chest physiotherapy was seen. The greater distance achieved is reflected in both the activities score of the SGRQ and the physical domain of the LCQ. It is perhaps surprising however that despite this improvement in exercise capacity no changes were seen in either spirometry (including small airways) or in muscle pressures as assessed by inspiratory and expiratory mouth pressures. The median age of the study population was 73 years and with increasing age, there is less airway reversibility, perhaps further limiting the opportunity for any benefit from chest physiotherapy on FEV<sub>1</sub> or FVC. Only one of the previous studies of physiotherapy techniques in bronchiectasis found a significant improvement in FEV<sub>1</sub> (0.08L using the Flutter device) but the authors suggested that although this was a statistical improvement and was unlikely to be clinically relevant. In view of the increased prevalence of bronchiectasis in those over 75 years of age and the increasing life expectancy of the general population worldwide, the sensitivity and utility of airways reversibility as a measure of treatment efficacy requires further investigation and may need to be reconsidered (Weycker D, 2005). However, the study described in Chapter Three involves only 20 patients and as such is not sufficiently powered to show changes in spirometry or small airways.

Although regular chest physiotherapy clearly has significant effects on functional measures, no significant changes were seen in either quantitative or qualitative sputum

bacteriology. Despite mobilisation and increased clearance of secretions from the airways, chronic colonisation of the bronchial mucosa persists. No change in exacerbation frequency was observed suggesting that patients remain vulnerable to repetitive insults to the airways and disease progression although again the size of the population studied and the duration of the study may have been insufficient to fully evaluate this outcome. Larger multicentre studies are needed to explore the effect of physiotherapy on bacterial colonisation, microbial load in the airways and exacerbation frequency.

The current study provides evidence for the clinical efficacy of regular chest physiotherapy in bronchiectasis but highlights major areas that still require research. In particular, studies of longer duration that can assess the impact of physiotherapy on exacerbation frequency and severity are needed and the efficacy of chest physiotherapy in combination with other airway clearance techniques requires considered evaluation. Other questions that need answered include whether the aetiology of bronchiectasis affects the efficacy of physiotherapy; whether there is an optimum technique for physiotherapy; how frequently and for how long should physiotherapy be practised and whether it provides additional benefits during acute exacerbations. The present study provides the initial evidence base from which these important questions need to be fully answered.

## **7.2 Successfully treating exacerbations to disrupt the vicious cycle**

Infective exacerbations are a significant cause of morbidity in bronchiectasis. Although there are no formal criteria specific to bronchiectasis that define an exacerbation, consensus expert opinion agrees that an exacerbation involves an acute deterioration (usually over several days) with worsening local symptoms (cough, increased sputum volume or change of viscosity, increased sputum purulence with or without increasing wheeze, breathlessness or haemoptysis) and/or systemic upset (Pasteur et al., 2010). Infective exacerbations have a negative impact on health related quality of life, absent days from work occur and hospital admission may be necessary. On a pathological level, acute bacterial infection in the airways is associated with increased neutrophilic inflammation and airway wall damage, and with repeated episodes perpetuating the vicious cycle of infection and inflammation in the airways, there may be progressive destruction of the integrity of the bronchial wall.

More frequent and severe exacerbations are associated with a progressive decline in lung function and a poorer health related quality of life (Martinez-Garcia et al., 2007) (Martinez-Garcia et al., 2005, Courtney et al., 2008). Although exacerbations have not been explored as an independent risk factor for mortality, a New Zealand study observed that 21% of 152 patients who had required a hospital admission for an exacerbation of bronchiectasis had died within the next 12 months (Roberts et al., 2011) and an American study of 43 patients with inpatient exacerbations of bronchiectasis found a hospital mortality of 9% and a one year mortality of 30% (Finklea et al., 2010).

Exacerbations are commonly managed with in the community, although such outpatient exacerbations are poorly quantified or indeed characterised in the literature (Kelly et al.,

2003). More severe exacerbations typically require management as an inpatient in hospital, often due to associated systemic sepsis or the need for intravenous antibiotic agents if these are unable to be administered in the community. There has been a decline in the number of inpatient admissions for exacerbations of bronchiectasis over the past few decades with a Finnish study observing 87 admissions per million of the population in 1992 compared with 143 admissions per million of the population in 1972 (annual reduction of 1.3% with a 5.7% annual reduction of the number of necessary inpatient days) (Saynajakangas et al., 1997). Various factors are likely to be responsible for this decline including better understanding of bronchiectasis, increased availability of preventative measures such as vaccination, development of more effective antibiotic agents and improved community health care services. Despite this, exacerbations necessitating inpatient management remain a significant problem. The average annual age-adjusted hospitalisation rate of US inpatient admissions between 1993-2006 was 16.5 per 100 000 of the population (Seitz et al., 2010) and more recently a study of 152 patients observed 307 admissions over a 12 month period (Roberts et al., 2011).

Despite the morbidity and mortality associated with exacerbations of bronchiectasis, little is known about how best to assess the effect of antibiotic treatment in the management of such exacerbations, to define a successful treatment outcome.

The aim of the study described in Chapter Four was to assess the effect of two weeks of intravenous antibiotic therapy for exacerbations on a variety of both clinical and laboratory endpoints including 24 hour sputum volume, FEV<sub>1</sub>, FVC, exercise capacity, systemic inflammation, sputum microbiology and health related quality of life. In

addition, the aim was to determine whether these outcomes were influenced by the pathogenic organism isolated.

Improvements were observed in 24 hour sputum volume, sputum bacterial clearance, systemic inflammation, exercise capacity and health related quality of life, independent of the infective organism. Four particularly responsive markers were identified: 24 hour sputum volume, qualitative sputum bacteriology, CRP and SGRQ score.

Sputum expectoration is the defining symptom of bronchiectasis and as such is a pertinent and non-invasive potential marker of bronchiectasis. Following 2 weeks of intravenous antibiotic treatment, all patients had less sputum production with 80% achieving  $\geq 50\%$  reduction in 24 hour sputum volume. Previous antibiotic studies have utilised sputum volume as a parameter of efficacy (Hill et al., 1988, Currie et al., 1990, Tsang et al., 1999b) and the current study supports this, finding 24 hour volume to be the most responsive marker. However, for 24 hour sputum volume to be reliable it is entirely dependent on patient compliance for accurate collection. Other more independent objective markers of efficacy are likely to be necessary.

Treatment of exacerbations with 2 weeks of intravenous antibiotic therapy had a positive impact on health related quality of life with 89.7% of patients having a clinically significant response in SGRQ score (greater than 4 unit change). A significant improvement was seen in each domain assessed by the SGRQ (symptoms, activities and impact) and the mean improvement of 13.8 units in total score was more than three times greater than the minimal important difference for change necessary with the SGRQ. Achieving a better score in the activities section of the questionnaire may reflect an improved prognosis in terms of mortality risk factors (Loebinger et al., 2009b).

Improving health related quality of life is a major goal of management in chronic disease and in non-cystic fibrosis bronchiectasis it's importance has been recognised since the early studies of antibiotic treatment for exacerbations that reported various features such as improved wellbeing and symptoms, fewer days confined to bed and fewer days absent from work as outcomes of successful treatment (Hill et al., 1988, Currie et al., 1990, Davies and Wilson, 2004, Cymbala et al., 2005). Health related quality of life questionnaires have provided a powerful means to quantify such important outcomes and have been widely used in more recent studies to both assess treatment response and to compare interventions. A study of inflammatory indices in 18 patients receiving two weeks' intravenous antibiotic therapy for an infective exacerbation also observed an improvement in the patients' health related quality of life as measured by the Chronic Respiratory Disease Questionnaire and although this has not been validated for use in non-cystic fibrosis bronchiectasis, significant improvements were seen in three of the questionnaire subsections including the dyspnoea, emotional and mastery scores (Courtney et al., 2008). However, the utility of such questionnaires remains predominantly within the research setting as administration and analysis require specific resources and are time consuming.

The French mycologist Vuillenin used the phrase “*antibiosis (Greek: anti, against and biosis, life)*” to describe the activity of antibacterial substances. The term antibiotic was derived from this phenomenon by Waksman in 1942 and clearly explains the rationale for the use of such substances in the management of infection, to inhibit microbial growth (Waksman, 1947). Studies of exacerbations of bronchiectasis have all used antibiotics with the aim of limiting bacterial growth in the airways. In the current study,

the effect of intravenous antibiotic therapy on the infecting pathogen was assessed using qualitative sputum bacteriology. 78.1% achieved complete clearance of the infecting pathogen at the end of 14 days of intravenous antibiotics. This is similar to previous studies of intravenous antibiotics for exacerbations with one of the earliest studies using intravenous ticarcillin or intravenous and nebulised ticarcillin for patients infected with *Pseudomonas aeruginosa* achieving bacterial clearance in 76% (Pines et al., 1970). Later studies using intravenous antibiotics alone have also achieved similar clearance with a study of 9 patients treated with intravenous meropenem achieving complete bacterial clearance in 83% and more recently a study of 18 patients receiving intravenous antibiotics for 14 days achieved clearance in 72.2% (13 patients) at the end of treatment (Darley et al., 2000, Courtney et al., 2008). In the remaining 22.9% in the study in Chapter Four there is no information on whether there was a reduction in bacterial load as only qualitative cultures were carried out. However, these patients did show improvements in the 3 other key parameters identified in this study: 24 hour sputum volume (mean reduction 13.8mls), CRP (mean reduction 61.4mg/ml) and SGRQ score (mean reduction 14.8units). Further studies using both qualitative and quantitative cultures would be helpful. The current study described in Chapter Four did not follow the patients beyond the end of the 14 day treatment period and so it is unknown as to whether the patients achieving bacterial clearance subsequently became colonised or indeed reinfected with the same pathogen. Previous studies that have reviewed sputum bacteriology subsequent to the immediate end of treatment of the exacerbation have found variable rates of relapse between 15% (Courtney et al., 2008) and 47-52% (Davies et al., 1983, Pines et al., 1974). Reinfection of the airways poses many questions such as the type of infecting pathogen, resistance pattern of the



infecting pathogen, the burden and impact of the pathogen in the airways and on the vicious cycle of infection and inflammation and further studies are needed to address these issues.

The current study used systemic markers of inflammation to assess treatment response and observed a 9 fold fall in C reactive protein (mean fall 59.5mg/L), with 62.5% having a  $\geq 75\%$  improvement following antibiotic therapy. The other markers of systemic inflammation measured observed were WCC and ESR. Although a significant improvement was seen in WCC (1.6 fold fall with a mean fall of  $4.0 \times 10^9/L$ ), the mean WCC at the start of the exacerbation was within normal range and the reduction following antibiotics was small, suggesting WCC is a less relevant marker of treatment response. The mean ESR also improved (1.8 fold fall with a mean fall of 17.7mm/hr) however, only 7% of patients achieved  $\geq 75\%$  reduction in ESR. The study also assessed the effect of treatment on exercise capacity and lung function. In keeping with the improvement in health related quality of life (particularly the activities domain of the SGRQ), exercise capacity improved by 1.2 fold with a mean rise of 54.1m and 36% of patients increased their distance in the ISWT by  $\geq 50\%$ .

There was no significant improvement in FEV<sub>1</sub> however, and only a small 1.06 fold rise in FVC (mean rise of 144ml). Similar findings were made by the authors of a previous study who observed that following 2 weeks of treatment with oral amoxicillin there were small improvements in FEV<sub>1</sub> (mean improvement 8mls) and FVC (mean improvement 180mls) but these changes were not felt to be clinically useful (Hill and Stockley, 1986). Interestingly, in the subanalysis of patients infected with pathogens other than *Pseudomonas aeruginosa*, an improvement in FEV<sub>1</sub> (from 1.32L to 1.51L)



and FVC (from 2.25L to 2.58L) was observed, perhaps reflecting the complete bacterial clearance achieved in this group. The utility of FEV<sub>1</sub> and FVC as markers of effective treatment is debatable and a recent review article of exacerbations in bronchiectasis noted FEV<sub>1</sub> to be a less sensitive measure in acute exacerbations, particularly in contrast to cystic fibrosis bronchiectasis (Chang and Bilton, 2008).

Previous antibiotic studies have compared antibiotic dose and duration in patients infected with either a variety of pathogens or in those exclusively infected with *Pseudomonas*. The aim of the current study was to explore how best to assess treatment efficacy in exacerbations irrespective of infecting pathogen.

The duration of antibiotic therapy for treatment of exacerbations of bronchiectasis in this study was 14 days. The optimum duration of treatment is not known and there are no randomised controlled trials of duration of antibiotic use in exacerbations. Studies have included exacerbations using antibiotics for between 5 to 28 days and good bacterial clearance has been proven in exacerbations managed with antibiotics for 7-14 days (Pines et al., 1967, Pines et al., 1970). Current national guidelines advise that treatment is for 14 days based on consensus opinion but clearly further studies evaluating the optimum period of treatment are needed (Pasteur et al., 2010). All patients included in the study that were infected with *Pseudomonas aeruginosa* or Gram negative organisms were treated with two anti-pseudomonal antibiotics to reduce the chance of developing antibiotic resistance. Patients infected with other pathogens were treated with monotherapy using intravenous ceftriaxone. The optimum therapeutic agents for treating exacerbations are unknown, with previous studies of antibiotic treatment for exacerbations finding variable results with different agents but no clearly

superior antibiotic has been identified. Consensus expert opinion currently recommends that treatment is initiated according to in vitro sensitivities of the most recent infecting organism for each individual patient until the causative organism is identified from an acute sputum sample cultured at the start of the exacerbation (Pasteur et al., 2010).

Many other questions regarding the optimal management of exacerbations remain unanswered including the optimal route of antibiotic administration; whether dual antibiotic treatment is superior to monotherapy regardless of the infecting pathogen; the role of adjunctive therapies and the optimal duration of treatment. Exacerbations are a further insult to the already damaged airways in bronchiectasis and a potent stimulus of the vicious cycle of inflammation and infection. The current study identified multiple parameters that may be useful for defining a successful treatment response in adults with exacerbations of bronchiectasis and in particular found four highly responsive markers of successful treatment: 24 hour sputum volume, qualitative sputum bacteriology, CRP and SGRQ score. These outcome measures remained robust markers of successful treatment irrespective of the infecting pathogen and may have an important role as further strategies to limit exacerbations and their impact on the vicious cycle of inflammation and infection in the airways are investigated.

### **7.3 Reducing the chronic bacterial burden in the airways**

Fundamental to the hypothesis of disease pathogenesis in bronchiectasis is chronic bacterial invasion and colonisation of the airways persistently stimulating an exaggerated neutrophilic inflammatory response (Cole, 1986). Previous studies have found 64% to 79% of patients with bronchiectasis to be colonised with bacterial pathogens in the airways when clinically stable (King et al., 2007, Angrill et al., 2002, Pasteur et al., 2000) and bacterial density in the airways has been shown to correlate with the degree of airways inflammation (King et al., 2007, Angrill et al., 2001, Hill et al., 2000).

Long term antibiotic therapy could limit chronic bacterial infection in the airways thus reducing neutrophilic inflammation and improving morbidity. Targeted delivery of aerosolized antibiotics directly to the airways may have significant benefit with minimal adverse systemic effects. The aim of the randomised controlled trial reported in Chapter Five was to assess the efficacy of continuous nebulised gentamicin therapy over one year in patients chronically infected with a variety of pathogens in their airways and to assess whether any treatment effects could be sustained over a three month treatment free follow up period.

The study was unique to include patients infected with a variety of pathogens as previous randomised controlled trials of nebulised antibiotic therapy have been conducted only in patients colonised with *Pseudomonas aeruginosa* (Orriols et al., 1999, Drobic et al., 2005, Barker et al., 2000). Although chronic *Pseudomonas aeruginosa* infection is associated with more severe bronchiectasis in terms of the radiological extent of disease, impaired lung function and a poorer health related quality

of life (Miszkiel et al., 1997, Ho et al., 1998, Wilson et al., 1997b), chronic infection with other pathogens is still associated with marked neutrophilic airways inflammation and significant morbidity with exacerbations and poor health related quality of life (Wilson et al., 1997b, Angrill et al., 2001, Hill et al., 2000, Martinez-Garcia et al., 2005). Inclusion of patients chronically infected with a variety of pathogens represents a far greater section of the bronchiectasis patient population, and provides an evidence based treatment for more patients.

A significant reduction in bacterial density was seen after 3 months of nebulised gentamicin, an effect sustained throughout the 12 months' of treatment. In the gentamicin group pathogen eradication was achieved in 92.8% of patients colonised by pathogens other than *Pseudomonas aeruginosa* and in 30.8% of patients colonised with *Pseudomonas aeruginosa*. In those not achieving complete eradication of pathogens, there was still a significant reduction in bacterial density seen from a median  $8.06\log_{10}\text{cfu/ml}$  at baseline to  $6.17\log_{10}\text{cfu/ml}$  at the end of treatment. These results are similar to a previous study of 4 weeks of inhaled tobramycin solution in patients colonised with *Pseudomonas aeruginosa* that achieved eradication in 35% and observed an overall reduction of  $4.54\log_{10}\text{cfu/g}$  in sputum bacterial density (Barker et al., 2000). A previous study of airway bacterial colonisation in clinically stable patients with chronic bronchitis and bronchiectasis found that with lower bacterial loads there was significantly lower neutrophilic inflammation as evidenced by lower levels of myeloperoxidase activity, lower levels of interleukin-8, lower levels of leukotriene B4 and lower levels of elastase (Hill et al., 2000). In the gentamicin group in the study in Chapter 3, as bacterial density in the sputum reduced, reductions in the concentration of

myeloperoxidase were seen with levels reducing from 51.6units/ml to 13.8units/ml at the end of 12 months' treatment. A similar effect was also seen in free elastase activity reducing from 3.6units/mg to 0units/mg at the end of 12 months' treatment. These findings provide support for the hypothesis of the vicious cycle of inflammation and infection thought to occur within bronchiectatic airways; with a reduction in bacterial density, neutrophilic inflammation in the airways also significantly reduces.

Despite good improvement in bacterial clearance, there was no significant change in systemic inflammation between the groups at the end of 12 months' treatment. Although previous work has shown that increased levels of WCC, CRP and ESR may be present in patients with stable disease and correlate with some disease characteristics they have not been shown to correlate with sputum bacteriology (Wilson et al., 1998) and a separate study demonstrated the association between systemic and airways inflammation to be poor (Angrill et al., 2001).

The effect of reducing bacterial density and neutrophilic airways inflammation was evident in other clinical parameters studied. There was less sputum purulence with gentamicin treatment (66.7% of patients had purulent sputum at the start of treatment compare with 8.7% at the end), improved exercise capacity (the median distance achieved at the start of treatment was 350m compared with 510m at the end) and fewer exacerbations with a median number of 0 over the 12 month period compared with 1.5 in the saline group. With these significant reductions in sputum bacterial density and inflammation and improved clinical features, significantly more patients in the gentamicin group had a clinically relevant improvement in health related quality of life

as assessed by both the LCQ and the SGRQ, arguably the most important goal of management in chronic disease.

This study provides evidence for the efficacy of regular nebulised gentamicin therapy in patients with chronic bacterial infection of the airways. Gentamicin was selected as it is broad spectrum for the pathogens seen in non-cystic fibrosis bronchiectasis and has previously been shown to achieve good sputum concentrations with a previous study demonstrating that a single dose of 160mg of nebulised gentamicin, achieved good sputum concentrations (97.2ug/g sputum) two hours following nebulisation. Nebulised gentamicin was reasonably well tolerated with few adverse effects which contrasts with studies of other nebulised antibiotics such as tobramycin which administered over a four week period was associated with 70% of patients reporting at least one respiratory symptom and an overall increased incidence of reported wheeze, cough, chest pain and dyspnoea (Barker et al., 2000). A later study of 14 day cyclical nebulised tobramycin administered for 12 weeks had adverse events related to treatment in 22% of study patients (Scheinberg and Shore, 2005). Despite significant benefits with nebulised gentamicin throughout the 12 months of treatment, it was disappointing that none of the treatment effects were sustained in the 3 month treatment free follow up period, with sputum characteristics returning to values similar to baseline. Only one randomised controlled trial of nebulised antibiotics has previously included a follow up period during which time eradication of *Pseudomonas aeruginosa* from the sputum was sustained, but this was only over 2 weeks follow-up period and it is not known whether this effect would be sustained over a longer follow-up period (Drobnic et al., 2005). Although the findings during the follow-up period of the nebulised gentamicin study are

slightly disappointing, they provide clear guidance for clinicians that for treatment with nebulised gentamicin to be effective, it needs to be continuous. There have been no previous long term studies of nebulised gentamicin in bronchiectasis and further studies to determine the optimum dose and regime are needed.

Ultimately, this study corroborates the hypothesis of the vicious cycle of infection and inflammation in bronchiectasis. It provides evidence for the efficacy of regular nebulised gentamicin with significant reductions in sputum bacterial infection, reduced airways inflammation, improved sputum purulence, improved exercise capacity, fewer exacerbations and a better health related quality of life in chronically infected patients with bronchiectasis, irrespective of infecting pathogen.

#### **7.4 Assessing response to treatment**

The aim of interventional strategies for bronchiectasis is twofold: to disrupt or halt the infective and inflammatory activity constantly occurring within the airways and to alleviate the persistent, debilitating symptoms of the disease. The previous studies in Chapters Three to Five have explored potential means of affecting the vicious cycle of infection and inflammation by facilitating the impaired mucociliary clearance mechanism, by limiting the impact of further insults to the airways with effective treatment of infective exacerbations and by attempting to reduce chronic airways infection. As with previous interventional studies in bronchiectasis, various markers have been used to measure the success of these strategies. Markers used in these studies have varied but have included clinical markers such as sputum volume, sputum purulence, lung function, exacerbation frequency, reported cough and dyspnoea severity scores as well as laboratory markers such as sputum bacteriology and sputum



inflammatory indices (1957, Cherniack et al., 1959, Hill et al., 1988, Stockley et al., 1984a, Currie et al., 1990, Elborn et al., 1992, Rayner et al., 1994, Tsang et al., 1999a, Cymbala et al., 2005, Martinez-Garcia et al., 2006). Despite these studies and mutually acknowledged treatment goals, there are few validated methods to allow clinicians to assess response to treatment. The markers used have been selected based predominantly on their utility in other chronic respiratory diseases such as cystic fibrosis and COPD but the majority have not been validated for use in bronchiectasis and their application and actual significance remains unknown. There is an urgent need to identify relevant, reliable, non-invasive and easily accessible markers to assess response to treatment in bronchiectasis.

The study in Chapter Six aimed to evaluate some of the measures that are used most frequently to assess interventions in bronchiectasis, to try and establish their actual relevance as outcome markers for a successful response to long-term treatment strategies. Parameters used in the studies described in Chapters Three and Five were evaluated using the SGRQ as a gold standard measure of treatment response for comparison. Independent markers identified as potentially useful in the assessment of a successful response to treatment included an improvement in the incremental shuttle walk test of  $\geq 50\text{m}$  and complete eradication of pathogens in the sputum. In addition, an improvement in sputum colour and a reduction sputum bacterial density were identified as potentially useful markers of treatment response.

Persistent bacterial infection of the airways is a diagnostic feature of bronchiectasis and reflects the perpetual nature of the vicious cycle of inflammation and infection in the airways. Patients who are chronically infected with potentially pathogenic



microorganisms in their sputum have a poorer health related quality of life (Wilson et al., 1997b) and may have a greater decline in lung function. Previous studies have demonstrated that with less bacterial infection in the airways, there is less airways inflammation limiting the locally destructive impact of the vicious cycle (Angrill et al., 2001, Hill et al., 2000). Limiting bacterial infection is a major goal of management and sputum bacteriology has therefore been utilised as a measure of treatment efficacy in many of the therapeutic interventional studies to date. The univariate analysis conducted in Chapter Four found a reduction in sputum bacterial density to be a marker of treatment response and complete clearance of pathogens from the sputum was a significant and independent predictor of a successful treatment response on multivariate analysis. With reduced bacterial infection in the airways, patients' symptoms improve. Chronic airways infection may limit the utility of qualitative microbial assessment in bronchiectasis despite its power as an independent predictor of treatment response and the role of quantitative bacteriology requires further validation.

The incremental shuttle walk test is a means of assessing exercise capacity. Exercise capacity is influenced by several factors including both respiratory health and systemic health. Lung function, bronchial secretions, respiratory muscle strength as well as general health status (including nutrition and the impact of any comorbidity) may all influence performance and increasing the distance completed in the test may reflect either direct or indirect improvements in these aspects of health. It is interesting to observe that despite the ISWT distance improving with chronic intervention, no parallel improvement in spirometry (FEV<sub>1</sub> and FVC) occurred. This is intriguing and suggests that exercise capacity may reflect systemic factors as well as respiratory factors. This

hypothesis is further supported in a study of the 6 minute walking test as a measure of exercise capacity in 27 patients with bronchiectasis (Lee et al., 2009). This study found the FVC and health related quality of life as measured by both the Short Form-36 (SF-36) and the SGRQ to correlate with performance in the 6 minute walking test. On further analysis, the SGRQ activities and symptoms scores as well as the number of bronchopulmonary generations affected by bronchiectasis emerged as independent predictors of performance in the test, leading the authors to conclude that health related quality of life had a stronger association with exercise capacity compared with physiological measures of disease severity (Lee et al., 2009). The relationship between the ISWT with both systemic and respiratory factors, as well as the influence of potential biases such as age and comorbidities requires further investigation. However, the emergence of the test as an independent predictor of treatment success in both models of the multivariate analysis in Chapter Six provides compelling support for an external validation study of the ISWT in bronchiectasis.

Lung function has been used in many of the interventional studies in bronchiectasis to date. However, it is evident both from the previous studies in Chapters Three, Four and Five together with the findings in Chapter Six that although FEV<sub>1</sub> and FVC may have statistical utility in response to acute interventional strategies the actual clinical impact of the changes observed may be more limited: the median change in FEV<sub>1</sub> with acute treatment was 0.09mls and with FVC 0.08mls. The lack of response of lung function in the studies reported in this thesis as well as other studies is initially surprising, particularly given the utility of FEV<sub>1</sub> and FVC in other chronic lung diseases such as cystic fibrosis. It may be that the older median age of patients with bronchiectasis in

these studies minimises the opportunity for airways reversibility. Different measures of lung function such as the lung diffusion capacity, total lung capacity or residual volume may offer more utility as markers of treatment response particularly as these measures have been found to be independently associated with mortality (Loebinger et al., 2009b). The lung diffusion capacity may be of further relevance with a longitudinal study in 61 patients over a median of 7 years observed a progressive median decline of 2.4% of predicted value per year (King et al., 2010). Recently, in other chronic inflammatory lung diseases such as cystic fibrosis and asthma, assessment of small airway function as a measure of disease has been explored. The Lung Clearance Index (LCI) is such a measure of lung physiology derived from multiple breath washout (MBW) tests. MBW tests involve the washout of an inert tracer gas from the lungs during relaxed tidal breathing- with each successive breath of the washout there is a fall in the peak concentration of the exhaled tracer (Robinson et al., 2009). In chronic inflammatory lung diseases factors that may contribute to airway narrowing such as mucus retention, inflammation and airway wall remodelling contribute to ventilation heterogeneity and washout takes longer. The LCI represents the number of times the volume of gas in the lung at the start of the washout must be turned over to effectively washout the inert tracer gas; with increasing disease severity, the LCI increases. The LCI has been proven to be more sensitive than spirometry in adults with cystic fibrosis (Horsley et al., 2008) and has proven utility in paediatric patients with asthma and cystic fibrosis (Gustafsson, 2007). To date, the role of MBW tests and the LCI has not been explored in non-cystic fibrosis bronchiectasis. Further studies are needed to investigate the relevance of different aspects of lung function in bronchiectasis to help assess its potential utility as a pertinent marker of treatment response.

It is disappointing that exacerbation frequency did not emerge as an independent predictor of a successful treatment response in either the univariate or multivariate analysis in the study in Chapter Six. Exacerbations are a significant source of morbidity and reducing frequency is typically a major goal of management. The analysis in Chapter Six may have been limited by the differing length of time in the two studies of chronic intervention and indeed the study reported in Chapter Five found that treatment with nebulised gentamicin lead to significantly fewer exacerbations and a longer time to first exacerbation than those who received nebulised saline. More studies of longer duration are necessary to accurately evaluate exacerbation frequency as a marker of successful treatment response.

Systemic inflammatory markers did not emerge as discriminators of response to long term treatment in the analysis in Chapter Six. However, in response to treatment of acute exacerbations as described in Chapter Four, there were significant reductions observed in mean total leukocyte count ( $3.7 \times 10^9$  cells/L), erythrocyte sedimentation rate (17.7mm/hr) and in C-reactive protein (59.5mg/L). It is likely that the significant improvement observed in these markers reflects the resolution of the systemic inflammatory response in an infective exacerbation. In stable bronchiectasis, the role of inflammatory markers is not yet established, with limited studies to date exploring the association of a variety of markers with airways inflammation or the extent of clinical disease (Lloberes et al., 1992, Wilson et al., 1998). Larger and longitudinal studies in stable bronchiectasis are needed to establish the role of systemic inflammatory markers, but at present their utility in response to long term treatments is unproven.

Sputum expectoration is a defining symptom of bronchiectasis and the volume expectorated has been used to assess bronchiectasis and its response to treatment since the earliest reported case studies of the disease. One of the first published studies of a non-surgical treatment strategy in bronchiectasis was the twice daily intratracheal injection of menthol and guaiacol with olive oil for an acute exacerbation (Grainger-Stewart, 1893). This treatment was reported to be a clinical success based on the observation that “*the sputum gradually diminished in amount*”. 24 hour sputum volume has since been used in several more formal studies both as a marker in assessing potential long term therapeutic strategies including inhaled steroids and long term antibiotics as well as assessing response to treatment in acute exacerbations (Cymbala et al., 2005, Tsang et al., 2005a, Currie et al., 1990). The analysis in Chapter Six did not however, include sputum volume as a potential marker of successful treatment response due to its potential limitations in chronic disease. Collection of sputum is wholly reliant on patient compliance which may be more difficult to depend on in long term studies of stable bronchiectasis. The analysis also included a study of successful response to regular chest physiotherapy. It is widely acknowledged that the aim of chest physiotherapy is to enhance sputum expectoration and therefore volume which conflicts with the overall management goal in bronchiectasis to improve symptoms by reducing sputum volume- a successful response to chest physiotherapy may not therefore be reflected at face-value in the assessment of sputum volume. However, the longer term effect of regular airway clearance in stable disease is unknown and it may be that with frequent practise the airways may become clearer and actual volume decrease. The role of 24hour sputum volume as a marker of treatment response requires further study.

There is a real and urgent need to establish pertinent, reliable and easily accessible markers for treatment response in bronchiectasis particularly as exciting advances are made in the development of new treatment strategies. At present, three key markers have been identified from the studies reported in this thesis: an improvement in the SGRQ of at least 4 units; an improvement of at least 50metres in the ISWT and eradication of bacteria from the sputum. External validation studies are needed.

## **7.5    Future studies**

1. The importance of the dysfunction of the mucociliary clearance mechanism in bronchiectasis is clear. The study in Chapter One has provided evidence for the efficacy of regular chest physiotherapy in enhancing the efficiency of mucus clearance from the airways but raises questions that need to be explored and answered in future studies: is there an optimum regime for patients?; is there any further benefit in stratifying regimes according to predominant type of bronchiectasis (cylindrical, varicose or cystic) or to the aetiology of bronchiectasis?; do other airway clearance mechanisms offer additional benefit when used with regular physiotherapy such as airways humidification and whether there is a role for pulmonary rehabilitation in bronchiectasis?
2. Infective exacerbations perpetuate the vicious cycle of infection and inflammation and are a major source of patient morbidity. Early studies suggested that high doses of antibiotic are necessary but it is unknown as to whether there is any benefit in duration of regime. Current consensus expert opinion suggests prolonged treatment courses of 14 days but it is unknown as to whether this confers any further benefit to a standard 7 day course of treatment

in terms of effect on sputum bacterial density, airways inflammation, systemic inflammation or on health related quality of life as well as the increased potential for adverse treatment effects to the prolonged course of antibiotics. This optimum duration of treatment of infective exacerbations requires formal study. The impact of multiple courses of antibiotics for recurrent exacerbations on antimicrobial resistance patterns has not been established and may be of great importance in the long term management of these chronically colonised patients, impacting on choice of antibiotic for treatment of exacerbations and the potential need for dual antibiotic therapy. This needs further exploration. Traditional management of exacerbations has been with either oral or intravenous antibiotic therapy. Furthermore, the role of adjunctive therapies in the management of acute exacerbations including physiotherapy, airways humidification and enhanced nutrition needs to be explored.

3. The importance of bacterial colonisation in bronchiectasis is a major therapeutic target. The impact of chronic infection with *Pseudomonas aeruginosa* on lung function, exacerbation frequency and severity as well as health related quality of life is evident from the literature but it is not known whether targeted eradication treatment for the first isolation of the pathogen is successful in terms of complete curative clearance or duration of remission from infection and the impact on airways inflammation, exacerbation frequency and health related quality of life. Studies are needed to address this important issue and to explore what the optimum regime for eradication could be in terms of drug delivery and duration of eradication treatment or treatment attempts. Similarly the effect of eradication regimes on other pathogens such as MRSA is unknown and should



be studied. The role of long term antibiotics is hopeful but many questions remain unanswered at a microbial level and longitudinal studies are needed to assess the impact not only on resistance patterns but also on virulence factors and adaptation of the bacteria such as biofilm formation.

4. It is clear that there is an urgent need for the validation of markers specific to bronchiectasis that can be reliably and easily used to both assess response to treatments and monitor disease progression. The goals of management of bronchiectasis and the aspects of the vicious cycle offer the most obvious targets and studies to date have highlighted the particular potential of both sputum volume and exercise capacity. These measures need formal validation studies.
5. Bronchiectasis was once termed an orphan disease. It remains a significant problem but little is known about the current incidence and the potential influence of environmental factors on prevalence. Formal population studies are needed to assess the actual incidence of bronchiectasis and its causes in order to try and understand more of the aetiology of this debilitating chronic condition to both gauge the impact of the disease on future healthcare provision and to attempt to address causative factors that can be influenced.



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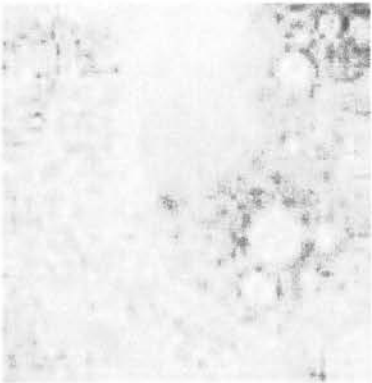
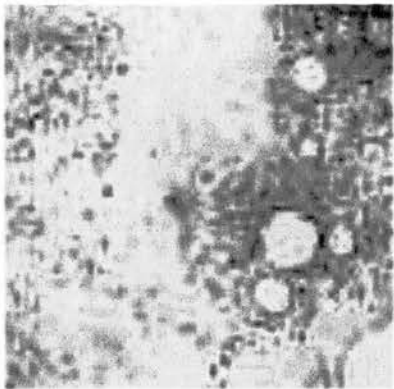
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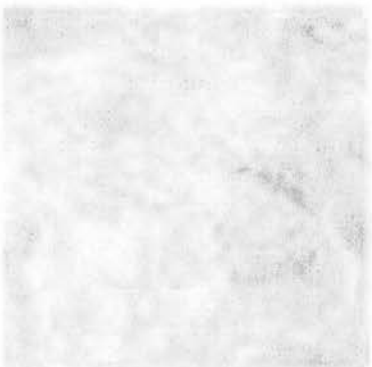
## **APPENDIX**

**9.1     Sputum colour chart**

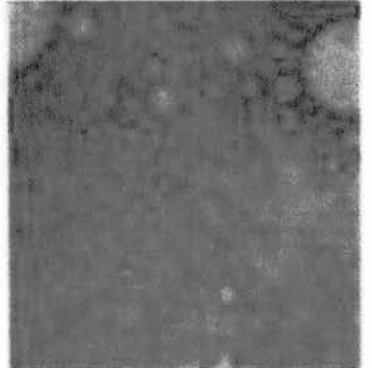
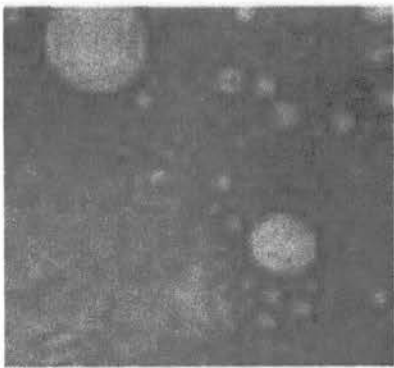
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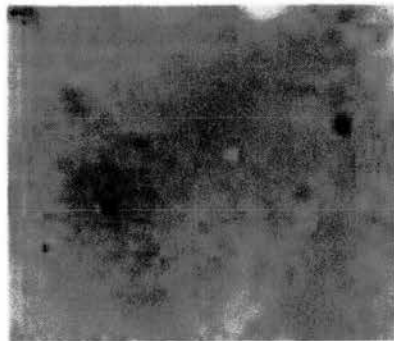
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## **9.2   Bronchiectasis diary card**

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**PUBLICATIONS ARISING FROM THIS THESIS**

### **9.3**    **Abstracts**

1. Long term nebulised gentamicin in non-cystic fibrosis bronchiectasis improves microbial load, exercise tolerance, exacerbation frequency and health related quality of life. MP Murray, JRW Govan, CJ Doherty, AP Greening, RD Gray, AJ Simpson, C Haslett, TS Wilkinson, JD Chalmers, AT Hill. Thorax 2009; 64:A103.
2. 24 hour versus early morning spontaneous sputum collection for routine microbiology in stable bronchiectasis. Murray MP, Doherty CJ, Govan JRW and Hill AT. ERJ 2009; 34(supplement3):412S.
3. A randomised crossover trial of chest physiotherapy in non-cystic fibrosis bronchiectasis. Murray MP, Pentland JL and Hill AT. ERJ 2009; 34(supplement3):413S.
4. Relationship of FEV<sub>1</sub> and exercise capacity in stable bronchiectasis. Murray MP and Hill AT. ERJ 2009; 34(supplement3):413S.
5. Can sputum colour predict bacterial colonisation, severity and quality of life in stable non cystic fibrosis bronchiectasis? Murray MP, Pentland JL, Turnbull K, MacQuarrie S and Hill AT. Thorax 2008;63(Supplement VII):A80.

6. Outcomes of exacerbations of non-cystic fibrosis bronchiectasis requiring intravenous antibiotics: influence of pathogenic organism. Murray MP, Turnbull K, MacQuarrie S and Hill AT. Thorax 2008;63(Supplement VII):A79.
7. The Leicester Cough Questionnaire is a useful marker of disease severity in non-cystic fibrosis bronchiectasis. Murray MP, Turnbull K, MacQuarrie S, Pentland JL and Hill AT. Thorax 2008;63(Supplement VII):A79.
8. 24 hour sputum volume is a useful marker in exacerbations of non-cystic fibrosis bronchiectasis. Murray MP, Turnbull K, MacQuarrie S and Hill AT. SMJ 2008; 53(4):57.
9. Outcomes of Exacerbations of Non Cystic Fibrosis Bronchiectasis Managed With 2 Weeks' Intravenous Antibiotic Therapy Murray MP and Hill AT. Thorax 2007; 62(Supplement III):A74.
10. Frequent outpatient exacerbations of non-cystic fibrosis bronchiectasis leads to impaired health related quality of life. Murray MP and Hill AT. ERJ 2007; 30(Supplement 51):438s.

11. Hospital admissions are linked to a poor health related quality of life in patients with non-cystic fibrosis bronchiectasis” Murray MP, Sutherland A and Hill AT. ERJ 2007; 30(Supplement 51):226s.

#### **9.4**    **Papers**

1.    A randomized controlled trial of nebulised gentamicin in non-cystic fibrosis bronchiectasis. Murray MP, Govan JRW, Doherty CJ, Simpson, AJ, Wilkinson TS, Chalmers JD, Greening AP, Haslett C and Hill AT. Am J Respir Crit Care Med 2011;183(4):491-499.
  
2.    Do timing and storage of sputum influence quantitative bacteriology in bronchiectasis? Murray MP, Doherty CJ, Govan JRW and Hill AT. J Med Microbiol. 2010. Jul;59(pt 7):829-33
  
3.    A randomized crossover trial of chest physiotherapy in non-cystic fibrosis bronchiectasis. Murray MP, Pentland JL and Hill AT. Eur Respir J 2009 Nov; 34:1086-1092.
  
4.    Validation of the Leicester Cough Questionnaire in non-cystic fibrosis bronchiectasis. Murray MP, Turnbull K, MacQuarrie S, Pentland JL and Hill AT. Eur Respir J 2009 Jul; 34(1):125-31.
  
5.    Sputum colour: a useful clinical tool in non-cystic fibrosis bronchiectasis. Murray MP, Pentland JL, Turnbull K, MacQuarrie S and Hill AT. Eur Respir J 2009 Aug; 34(2):361-4.

6. Assessing response to treatment of exacerbations of bronchiectasis in adults.  
Murray MP, Turnbull K, MacQuarrie S and Hill AT. Eur Respir J 2009 Feb;  
33(2):312-8